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SPORULATION AND VIABILITY OF *B. ANTHRACIS* IN RELATION TO ENVIRONMENTAL TEMPERATURE AND HUMIDITY

By

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INTRODUCTION

In India the problem of anthrax and its control remains as important as it has been for years. The matter of course concerns not India alone but all countries to which raw products of her animal industry are sent. Although at the present time a good deal of reliance is placed on vaccination, every step ought to be taken to prevent contact between the parasite and susceptible animals, and for this knowledge of the natural history of the parasite should be as clear as possible. Previously (Minett and Dhanda, 1941), one particular aspect had been considered, namely, the old hypothesis that the bacillus might find suitable conditions for multiplication in the outer world in soil and in water. Our experiments gave no support to this suggestion, but bearing in mind conditions in India, there seemed to be scope for further observations, now reported, in which emphasis would be laid on temperature and humidity relationships as affecting (*a*) the viability of the bacillus in the wet or dry state ; (*b*) sporulation, the property around which centres the viability of the organism in nature. It is necessary to test the organism's power of survival when sporulation is prevented by desiccation, unfavourable temperature or other factors, since should conditions later become favourable, sporulation will occur. Moreover, as long as the bacillus remains alive, there is a risk of dissemination of the disease by various agencies, such as insects and carrion-feeders.

METHODS

For most of the work the anthrax strain used, which was the same as in the previous soil experiments, was an old laboratory strain, still tolerably virulent, which had been isolated from an elephant. In a number of cases tests were also made with five strains isolated in France between 1940 and 1945, three from cattle and two from sheep. For the various tests, unless otherwise stated, " diluted anthrax blood " was used ; that is, blood from an animal, taken as soon as possible after death from anthrax, was diluted with fresh citrated blood, usually from a sheep, such that in tenfold serial dilutions of the mixture 1 c.c. 10^{-4} plated in agar gave some 200 to 400 colonies. At intervals as necessary, the blood under test was taken up in saline and similarly plated, the examination for spores being made from the first dilution after it had been heated at 60° C. for 30 minutes. Doubtful colonies were frequently checked by inoculating mice. A preliminary experiment suggested that for

demonstrating spores subjected to this moderate degree of heating a plain agar medium was as good as agar containing 2·5 per cent. ox blood or agar containing 1 per cent. sheep serum and 2 per cent. glucose. Nevertheless, the agar used was occasionally unsatisfactory in that it exhibited a break effect, a certain dilution giving large numbers of anthrax colonies and the succeeding dilution no growth. Partly for this reason, too much attention has not been paid to actual counts.

SPORULATION OF THE ANTHRAX BACILLUS

Sporulation under laboratory conditions

Towards the beginning of the century the conditions in which sporulation occurs received much attention. The following statement summarises much of the early information available as to the time required for spore formation at different temperatures. Temperature, of course, although a most important factor, is not the only one.

Author	*Temp. (F.)*	*Time (hours)*	*Material and method*
Koch (1876)	95	20*	Anthrax spleen ; micro.
	86	30	
	64–68	60–72	
Stephanidis (1899)	98	8	Agar cult. ; micro.
Weil (1899)	98–88	16	Blood ; cult. test.
	75	36	
	64	50	
Jacobsthal and Pfersdorff (1901)	98	6–8	Blood ; cult. test.
	72	22	
	61	62	
Preisz (1904)	98	6	Agar cult. ; micro.

* Not indicated as shortest time.

Koch's classical observations were made on unstained preparations of the tissue suspended in a suitable fluid, such as ox serum, on slides in a moist chamber. Below 64° F. sporulation was exceptional. Weil sealed a mixture of anthrax blood and broth in pipettes ; after they had been exposed at the various temperatures they were heated to destroy vegetative organisms, and the contents of the pipettes were then seeded to broth at 98° F. Jacobsthal and Pfersdorff added the blood to blocks of gypsum soaked in warm broth, and then tested for spores by heating and sowing in agar.

Migula (1897) refers to experiments with five anthrax strains which had been maintained for some time in the laboratory. At lower ranges of temperature (64° to 75° F.) some of these strains sporulated more slowly than others ; at 61° F., after 96 hours, three of the strains showed no spores and two a few only. Migula's results were presumably based on microscopical examination, and it

is also questionable how far they would apply to freshly isolated strains.

It was decided to test the rate of sporulation of the anthrax strains available at Mukteswar in the light of these early observations, firstly under appropriate laboratory conditions and next under conditions intended to simulate the natural.

Sporulation in a thin layer of liquid medium.—Two or three drops of citrated blood from a guinea-pig or mouse shortly after death were transferred to 50 c.c. sterile saline containing 1 per cent. heated ox serum. The medium was held in a 250 c.c. conical flask so as to form a layer not more than 1 cm. deep. It was usually warmed to the temperature required before the blood was added and the flask was frequently shaken to promote aeration of the medium. The temperatures usually selected were 90° F. and laboratory temperature ($\pm$ 70° F.), while in some experiments a control flask was kept in the cold ($\pm$ 6° C.). Samples for test were taken after eight to ten, 24 and 48 hours, sometimes later, and plated out before and after heating at 60° C. From a number of experiments it was clear that at 90° F. a small proportion of the bacilli had reached the heat-resistant stage of sporulation within eight hours, while at 70° F. sporulation was first evident after 24 hours. At both temperatures with continued incubation more and more organisms formed spores, so that in general by 24 to 48 hours at 90° F., and a day or two later at 70° F. great numbers of spores were present. There was some multiplication of the bacilli, especially at 90° F., and no doubt some germination of spores also. Owing to the errors involved, no attempt was made to construct curves showing the numbers of spores in relation to the total number of organisms.

In flasks, which shortly after seeding were placed in the cold, the viable count dropped very considerably within the first two or three days and was negative within ten to 23 days. In two experiments the flasks were kept between 55° and 60° F. In one of these the viable count was very low after 90 days and negative after 121 days; in the other it was practically negative after 55 days. No spores were formed in either case.

The above experiments were carried out with the elephant strain of anthrax. The five French strains were examined later. Among these there seemed to be variations in the capacity for sporulation, though in all cases some bacilli had completed the process within ten hours at 90° F. This variation appeared to be connected with the particular experiment and not with any intrinsic character of individual strains. There was no evidence that the variation was due to storage of the blood for some hours at $\pm$ 60° F., which was sometimes necessary when the blood-producing animal died at an inconvenient time.

Sporulation in thin layers of blood exposed to air at various temperatures and humidities.—In nature, blood from an anthrax

carcase may be splashed or spilt. The bacilli contained therein may then encounter environmental conditions varying in respect of atmosphere and humidity, thickness of the blood layer formed, and the kind of surface on which the blood comes to rest. To study the atmospheric conditions, diluted anthrax blood was spread on microscope slides and exposed at various temperatures from 98° to 70° F., and at relative humidities varying from 100 to 20 per cent. The amount of blood was generally 12 " drops " (about 0·35 c.c.), spread over a surface 2·5 cm. square, this area being demarcated by transverse strips of plasticine. The layer of blood was such that at 90° F. drying occupied from, say, four to 20 hours at relative humidities ranging from 20 to 80 per cent., while at 100 per cent. humidity the blood remained wet for at least 48 hours. The slides for receiving the blood were set up in containers (desiccators or mouse jars) of 2·2 to 3·2 litre capacity, the humidities required being obtained with sulphuric acid-water mixtures, as follows :—

R.H. per cent.	20	40	60	70	80	90
Acid per cent. (by weight) ...	57·7	48·0	38·6	33·7	27·1	16·4
S.G.	1·486	1·38	1·295	1·25	1·19	1·125

These are roughly the amounts of acid required for relative humidities at 93° F., but for convenience the same mixtures were used for all temperatures between 70° and 98° F. (A convenient formula for the acid-water mixtures is also given by Buxton and Mellanby, 1934.)

At intervals, between eight and 48 hours or more, the blood on the slide to be examined was thoroughly dispersed in 10 c.c. normal saline and titrated for bacilli and spores. To obtain closely reproducible results, it appears necessary that the containers should be of the same size and shape, that the surface area of the acid mixture should be constant and that the slides should be maintained quite level. There is also the difficulty that, after the atmosphere in the jars has been brought to the humidity required, the jars have to be opened for distributing the blood and for removing slides for examination. In the absence of special apparatus, these difficulties were dealt with as far as possible, and at times an experiment had to be repeated using a smaller or larger amount of blood. From an analysis of the results of eleven experiments, of which it is unnecessary to give details but which involved all six anthrax strains, it was concluded that, unless the blood has dried before the times stated, sporulation may be expected to occur at 98° F. by eight hours, at 90° F. by ten hours, at 79° F. by 18 hours and at 70° F. by 24 hours. Sporulation does not occur much below 70° F. With the same acid mixture the rate of drying of course increases as the temperature rises. The statement of times just given may be considered in rela-

tion to the following estimate of the rate of drying (hours) of blood of uniform thickness, at the various temperatures and humidities :—

R.H./T.	98° *F.*	90° *F.*	79° *F.*	70° *F.*
100		Over 48		
80	13 (7)	15 (8)	22 (3)	33 (8)
60	10 (7)	10 (7)	17 (3)	24 (7)
40	5 (5)	6 (8)	7 (1)	11 (8)
20	4 (1)	4 (1)	8 (1)	8 (1)

(Number of observations in brackets)

Thus, for instance, in seven observations the average rate of drying in air at 98° F. and 80 per cent. R.H., was 13 hours. According to the figures, in *still* air sporulation is unlikely to occur at any temperature when the relative humidity is below 60 per cent. It was also noted that bacilli which have survived drying can sporulate later when wetted and put at a suitable temperature. (See also Bongert, 1904*a*.)

Sporulation in exposed body tissues

The common statement that it is dangerous to lacerate anthrax carcases, while administratively sound, requires some amplification. It is certainly dangerous to do so when the air temperature is much above 70° F. If the temperature is much below 70° F. anthrax bacilli are unable to sporulate, they disintegrate more or less speedily, and are liable to be overgrown by contaminants. In fact, in blood which is fully exposed to natural contamination there is a race between sporulation and putrefaction, and the outcome depends at least in part on the prevailing air temperature.

The following observations were made on the effect of different air temperatures. At the time of sampling, the material to be examined was taken up on a cover slip, both sides of which were brought lightly in contact with the surface under test.

Experiment 1.—The livers were removed from two guinea-pigs dead of anthrax, and suspended in beakers over water at 90° F. or at 70° F. At 90° F. sporulation occurred on the exposed surface of the liver within 24 hours ; at 70° F. from the 48th hour only.

Experiment 2.—A goat just dead of anthrax had its neck skin cut and the jugular vein punctured. The prevailing air temperature was 66° to 71° F. Anthrax bacilli, but no spores, were present in the neck blood, when examined 24 hours later. Examination after 68 hours showed contaminants only. Incidentally, a pure culture of anthrax was obtained from the skin of this goat after five days, by which time the carcase was quite putrid.

Experiment 3.—A goat dead of anthrax was treated as in the last experiment. Some blood was collected in a watch glass which was enclosed in a petri dish ; in addition, 15 drops of the blood were

spread on each of four slides, of which two were exposed and two covered in a petri dish. The watch glass and slides were left by the carcase, which was kept in a small shed in air, the recorded temperature of which just above the carcase ranged from 62° to 66° F. (mean during week, 63·4° F.). The results were as follows: on the neck wound anthrax bacilli were numerous at first, but by 101 hours they could not be recovered in culture. Spores were not encountered up to the last examination at 129 hours. On the exposed slide the blood had become sticky after 30 hours, but no spores were present up to 57 hours. On the covered slide an examination after 129 hours, when the blood was still quite wet, showed great numbers of spores. In the blood in the watch glass a few spores were present after 78 hours and these increased in numbers up to 129 hours.

Experiment 4.—In this and the next two experiments, animals were examined in pairs in an attempt to show more clearly the effect of differences in air temperature. Two guinea-pigs shortly after death from anthrax had the neck skin reflected and vessels severed. One was put at 90° F., the other at 70° F. In the former abundant spores had formed in the oozing blood within 24 hours; in the latter repeated cultural examination up to 120 hours showed no spores. Up to 48 hours anthrax bacilli were abundant but later were overgrown by contaminants and could no longer be recovered.

Experiment 5.—Two goats one hour after death from anthrax had the skin and neck vessels severed. The blood ran over the iron tray on which the carcase was lying, and some of it was also collected in petri dishes which were then closed and left on the tray. One carcase was placed in a small room, the temperature of which during the first 15 hours was about 90° F. and then fell to 80° F. The relative humidity was gradually raised from about 40 to 60 per cent. The other carcase was exposed in a shed, the temperature of which ranged from 62° to 66° F. The results were as follows: at 90° to 80° after 17 hours abundant sporulation had occurred in the petri dish and slight sporulation in the neck blood. The blood on the tray was rich in bacilli, but spores were absent. Re-examination seven hours later gave the same result. At 62° to 66° F. spores were not found at tests made from the three sites (neck, dish, tray) after 24, 29, 48 and 96 hours. Bacilli, however, were present in large numbers for at least 48 hours and were mixed in culture with discretely growing contaminants.

Experiment 6.—Another pair of goats, one hour after death from anthrax, had the skin and neck vessels severed, the still liquid blood spurting over the neck and tray. One goat was at once put in the small room at 90° F. for the first 24 hours and at 80° F. for the next 21 hours, the relative humidity throughout being at 50 per cent. The other goat was exposed in air maintained at about 70° F. for the first 48 hours, after which it was placed in the room at 80° F. for the following 21 hours. Blood from both goats was taken into petri dishes and the lids immediately replaced; blood

from the goat at 90° F. was also taken into small dishes made of iron, copper, and zinc, the dishes being tilted so that the blood ran towards one corner to form a layer up to about 2 cm. thick. The blood in all these dishes clotted, and the clot was then sliced up and chopped with saline. The various dishes were left with the respective carcase. The 90° tray was of iron with some incomplete tar painting, the 70° tray was of rusted iron. The results of this experiment were as follows: with the goat at 90° F. spores had formed within 17 hours on the neck wound, in the blood in the various dishes and on the tray. The numbers at the different sites appeared to have increased during the next seven hours and were relatively high in the neck blood and in the petri dish, but sporulation was somewhat retarded in the metal dishes. With the goat at 70° F. no spores were present after 24 hours but by 44 hours they were present in small numbers on the neck wound and in the petri dish. Spores appeared in small numbers in the blood on the tray after 68 hours only the carcase then being at 80° F. Contaminants in appreciable numbers began to appear on the plates from 44 hours onwards.

The common occurrence of bacteria that are antagonistic to *B. anthracis* has long been known, while Schipp (1906) has also shown that filtered tissue juices from putrid bodies are highly bactericidal for the anthrax bacillus. In my experiments the fact that sporulation sometimes occurred slowly in blood taken from the carcase and exposed alongside it but at the same time largely protected from desiccation and contamination, contrasts sharply with the inhibition of sporulation at sites exposed to contamination. Mennucci (1934) has made a similar observation.

Minimum moisture requirements for sporulation in soil

As anthrax bacilli can live for a long time in dry soil (see below), it was of some interest to determine how much water has to be added to it to permit sporulation. Two experiments were made, in which undiluted anthrax blood was dried, powdered and then incorporated in finely divided, chemically dry, sterile soil in corked tubes. To some of them, varying amounts of sterile water were added. The tubes were placed at 90° F. for 48 hours, when the soil was "washed" and examined for spores. Duplicate series of tubes were prepared for moisture determinations.

The conclusion from the two experiments was that sporulation is possible in *sterile* soil when its content of added water reaches about 3 per cent., but not when it is 2 per cent. or less. In these estimations allowance has been made for the blood added to the soil, since the loss of weight when the soil is burnt for moisture determination will be due both to water and organic matter. As a corollary, the moisture content of 12 samples of "dry" soil dug from three plots at Mukteswar during May varied from 2·8 to 9·0 per cent. (mean 5·365). The samples included unestimated amounts of organic matter, as well as bound water.

Viability of spores in water

In a previous paper, Minett and Dhanda (1941) gave references to the literature on the viability of anthrax bacilli and spores in water. The strong resistance in general of spores includes the particular case of anthrax spores immersed for long periods in water. The matter is not quite simple. It seems that anthrax spores survive in sterilised water longer than in unsterilised, and that survival is likely to be shorter at 95° F. or even at 64° to 68° F. than at somewhat lower temperatures. In garden earth saturated with water, Sirena (1906) noted survival of anthrax spores for 14 years. Observations on the survival of spores, estimated at 500,000 per c.c., in sterile water over a deposit of soil, were made at Izatnagar from December, 1944, to January, 1947. Four-ounce bottles, nearly filled with the water, were sealed and kept partially immersed in a shaded pond. The general result was that during two years there was no sign that the number of viable spores in the water had declined.

The mean monthly temperatures (° F.) of the pond water during the first year of observation are tabulated below. The means were from morning and evening readings taken every two to four days.

Dec.	*Jan.*	*Feb.*	*Mar.*	*Apr.*	*May*
56·1	54·1	56	64·1	73·1	79·1
64	61	64·7	75·7	81·4	86·6
June	*July*	*Aug.*	*Sept.*	*Oct.*	*Nov.*
84·7	83	84	82	76·3	63·4
91·7	87·5	91·6	86·3	81·9	71·1

VIABILITY OF ANTHRAX BACILLI

Viability in dry blood at various temperatures and humidities

Possibly the first real contribution to this subject is that of Momont (1892), who stored anthrax blood in very thin layers *in vacuo* or in air in tubes at 61° to 72° F. and at 91° F. He noted survival up to 60 days at the lower temperature and for rather less at 91° F. Viability was tested by incubating the tubes after the addition of broth. Bongert (1904*b*) in similar experiments obtained survival for 35 to 50 days and showed by agar plating that death of the dried bacilli occurred gradually. The importance of the conditions of drying and storage in viability tests is now more fully appreciated (Rahm, 1945). In my experiments, diluted anthrax blood in amounts of five to 12 drops was spread on slides and subjected to different atmospheres. The results may be considered as a whole and are as follows.

Relatively rapid drying is more destructive than slower drying when the difference in rate is due to differing humidities of the

atmosphere. Thus, when five drops of blood in a thin layer are exposed at 30 per cent. R.H. at laboratory temperature ($\pm$ 65° F.), drying may be complete as early as three hours, while in the same conditions at 80 per cent. R.H. drying may take upwards of eight hours. In both cases, drying causes considerable destruction of bacilli, but at 80 per cent. R.H. the proportion of bacilli surviving is almost invariably greater than at 30 per cent. R.H. It must here be noted that the term " drying," as used in the everyday sense, is a relative one. That is to say, films that are dry to the naked eye after they have been exposed at 80 per cent. R.H. contain more moisture than ones similarly exposed to a 30 per cent. atmosphere. With continued exposure at 80 per cent. R.H., the numbers of survivors decline at a more rapid rate than is the case with the survivors of the 30 per cent. R.H. exposure. At this lower R.H. survival of a few organisms was noted up to 30 days in one experiment.

When the observations are extended to include higher temperatures (98° or 90° F.), the issue is likely to be complicated by sporulation. In fact, the tendency for this to occur sharply reduces the practical value of observations on the viability of the bacilli. In cases at the higher temperature where drying occurs before sporulation can be completed, comparison with room temperature shows more prolonged as well as a higher level of survival at the lower temperature, especially at lower humidities. At 98° F. also, a lower humidity (say 20 per cent.) is more favourable to survival than one of 50 per cent. Thus, in two experiments considerable numbers of bacilli were surviving at laboratory temperature at 20 per cent. R.H. after 60 to 90 days, whereas at 90° and 98° F. at the same humidity very few bacilli survived for this time. The longer survival compared with the 30-day survival in the previous experiment mentioned above, was probably due to the layer of blood being twice as thick.

In two experiments blood was dried quickly (three hours or less at 90° F. and 20 per cent. R.H.) and slowly (seven hours or more, mostly exposed to the air of the laboratory). The quick-dried slides were then kept at 90° F. and at room temperature (62° to 67° F.), at both temperatures in dry (20 per cent. R.H.) as well as in damp (80 per cent. R.H.) conditions. The slow-dried slides were similarly distributed. This experiment provided the following conclusions. Storage under moist conditions is quickly fatal to anthrax bacilli, none surviving beyond ten days. This was so whether the storage was at 90° F. or at room temperature and irrespective of whether the previous drying had been rapid or slow. When stored under dry conditions, the result depends on the temperature of storage. At 62° to 67° F. some bacilli live for over 40 days ; at 90° F. the bacilli die more quickly than this, but the rate seems to depend on whether the blood has been dried quickly or slowly. Bacilli which survive quick drying can remain viable for some time even at temperatures as high as 90° F. To sum up, the conditions

which are relatively optimal for survival of anthrax bacilli in dried blood are drying which is not very rapid, followed by storage in a dry atmosphere at a low temperature.

Viability in dry blood under more natural atmospheric conditions

It was intended to expose blood at Izatnagar at three different seasons, *viz.*, December, March and June, but it was only possible to carry out the December exposure. One drop of fresh undiluted anthrax blood was deposited at the bottom of small tubes, 25 × 8 mm. The blood was gently spread over the bottoms of the tubes, where it promptly clotted. Two such series were prepared from two different guinea-pigs, and blood was also spread on slides. The unplugged tubes and slides were dried over $CaCl_2$, drying of the blood on the slides taking one to six hours according to thickness. The specimens were placed in a sheltered spot in a garden beneath a wire cage containing the necessary thermometers and covered by a sack as protection from sun but not from wind. Both tubes and slides were 6 inches from the ground and were freely exposed to the air except for a single layer of muslin to avoid dust.

Detailed results are omitted. The drying process lowered the viable count in the tubes to about 10 per cent. of the original, which was estimated at 9·45 million bacilli in the drop. After 20 to 25 days in the case of the slides all the bacilli which had survived drying were dead. With the tubes the numbers of living bacilli had fallen to a few thousands after 13 to 20 days but persisted in very small numbers up to 108 days. Frequent checks throughout the experiment revealed no spores. The longer survival of a few bacilli in the tubes was probably due to the more restricted exposure to atmospheric moisture. The air temperature and humidity during this experiment were as follows :—

Month	*Mean daily temperature* (*)	*Relative humidity, per cent.*	
		10 *a.m.*	4 *p.m.*
December	63·5	66	42
January	60·7	79	50
February	66·6	72	39
March	77·3	50	27

(*) From four daily readings : maximum and minimum, dry bulb at 10 a.m. and 4 p.m.

Influence of various types of surface on viability

Six to eight drops of saline containing fresh guinea-pig anthrax blood were evenly applied to the substrate over an area 1 cm. square and put at 50° to 60° F. or 90° F. or both, in a vessel with water to keep the blood wet for 48 hours. The blood was then serially plated, unheated in the case of the low temperature exposure, and after heating at 60° C. following the 90° F. exposure.

Surfaces which proved to be non-bactericidal, and on which spores were readily formed, were : lead, glass, polished stone, soil, wool and feathers. With hair, straw and bran sporulation for some reason was delayed. Bactericidal surfaces were : iron, zinc, copper, brass, limewash, leather and lead paint. On all the above surfaces tests were made at both temperatures. Spores were also readily formed on tin, bone, wood, green leaf, filter paper, cloth and india-rubber. With aluminium, tar and rope results were rather irregular but there was usually a distinct bactericidal effect at both temperatures.

In these experiments metal surfaces were clean and polished. Wood, bone and leather, being absorbent, were not very satisfactory to work with, and the apparent bactericidal action of leather may have been due to the tendency of the surface to dry off. Wool, hair, feathers, straw and rope—all finely chopped—filter paper, bran, fine soil, limewash and tar, were spread in thin layers on glass slides. Where possible, all the materials were previously sterilised by autoclaving or hot air.

Viability in soil

Dry soil.—In July, 1945, at Mukteswar, eight drops of diluted anthrax blood were mixed with fine soil and spread on slides over areas 2·5 cm. square. As controls, the same amounts of blood were spread over similar areas on polished stone and glass. The specimens were kept in a dark laboratory cupboard in a desiccator at 40 per cent. relative humidity. Drying was slow, being complete after 48 hours. Repeated tests of the dry material showed no spores. Titration after nine days' storage gave the following numbers (thousands) of anthrax bacilli on the soil, stone and glass : 187, 112 and 107. After 340 days the numbers of living bacilli had declined to 560 on stone and 400 on glass ; after 478 days to 45 on stone, glass being then negative. After 634 days (April, 1947) the stone was negative, whereas nearly 5,000 anthrax colonies grew from the soil. Two of these set up anthrax in mice ; also the surviving bacilli were still capable of sporulating when suspended in saline at 90° F. The specific gravity of the acid mixture in the desiccator had changed during the 634 days from 1·38 to 1·47, *i.e.*, the relative humidity from 40 to near 20 per cent. The air temperature in the cupboard was taken almost daily ; mean monthly readings varied between 70° F. in the summer and 54° F. in winter, except for a fall in January, 1947, to 48·6° F.

The prolonged survival of the bacilli on dry soil is noteworthy, while the longer survival on glass compared with the previous experiments is presumably due to the almost unvarying dryness of the atmosphere. The result may be compared with that of Heim (1905), who found that the bacilli in anthrax blood dried on silk threads and stored over $CaCl_2$ lived for nearly two and a half years. Comparison may also be made with the work of Watts (1945), who showed that *Str.*

agalactiae in dried milk showed optimum survival, actually for over three years, at laboratory temperature in air at 15 to 25 per cent. relative humidity.

Wet soil.—An experiment of January, 1947, at Izatnagar was designed to test viability under more normal conditions, *i.e.*, when the soil is damp or wet and when exposure occurs at naturally varying air temperatures. Two c.c. diluted anthrax blood were mixed with 10 g. of dry soil in tubes (2·5 cm. diameter), to constitute " damp " soil ; for " wet " soil 4 c.c. water was also added. The damp soil was estimated to contain 4,000,000 anthrax bacilli per gramme. In some cases the soil was sterilised in others unsterilised. To a few tubes of unsterilised soil spores were added instead of bacilli. The tubes were exposed in air at below 70° F., and at intervals 1 g. of damp soil and the equivalent amount of wet soil were plated in agar. The results indicated survival of bacilli in the damp soil for eight and probably 20 days with the sterilised and for eight days with the unsterilised samples ; in the wet soil for 38 days with the sterilised and 20 days with the unsterilised samples. Examination for spores was confined to the first 20 days and results were negative. As expected, it was easier, both by culture and by mouse inoculation, to reisolate the spores from the soil after it had been heated at 60° C.

Anthrax bacilli in water

A few drops of blood containing anthrax bacilli were added to paper-filtered unsterilised water from a cattle trough ; some of the water was then kept at 50° to 57° F. and some at 90° F. In the first case the bacilli died within five days and in the second a fair proportion sporulated within that time. Two separate experiments gave the same result. In a further experiment the water was autoclaved before the blood was added. Here also at the cool temperature the bacilli declined in numbers, but many of the survivors sporulated when the water was transferred to 90° F. These results conform with the findings of others.

Temperature of natural water in North and South India.—In amplification of the few observations just mentioned, some records were taken at Izatnagar (United Provinces) and Sitarampet (Hyderabad, Deccan). These relate to two small ponds, varying in depth up to 9 and 14 inches according to season ; temperatures were taken every few days just below the surface and near the bottom. The Izatnagar water was shaded during May and June. The figures given are the monthly means (° F.) of temperatures recorded at 10 a.m. and 4.30 p.m., for one year, May, 1944, to April, 1945. Mean surface temperatures were usually up to 2° F. higher than those shown.

Survival of anthrax bacilli in the unopened carcase

Although there are a number of previous observations on this point, the opportunity was taken to collect a few more details, with

	Jan.	*Feb.*	*Mar.*	*Apr.*	*May*	*June*
			Izatnagar			
A.M.	52·3	56·3	63·5	70·3	73·9	79·3
P.M.	61·3	68·0	76·1	84·2	80·6	82·4
			Hyderabad (Deccan)			
A.M.	71·6	76·6	78·8	81·0	87·8	80·6
P.M.	80·6	90·2	95·0	103·0	98·6	93·2
*Max.	85·3	91·8	98·8	99·5	105·0	96·3
Min.	59·4	60·5	67·9	74·5	82·0	76·3

	July	*Aug.*	*Sept.*	*Oct.*	*Nov.*	*Dec.*
			Izatnagar			
A.M.	84·7	86·9	85·1	72·7	60·8	52·3
P.M.	93·2	96·8	95·0	85·1	72·5	63·5
			Hyderabad (Deccan)			
A.M.	79·7	82·4	84·2	84·6	78·8	71·8
P.M.	83·8	90·2	89·6	91·4	85·1	77·9
*Max.	85·5	88·4	87·2	86·9	84·6	85·8
Min.	73·4	73·1	72·5	70·1	62·4	55·6

* Mean maximum and minimum air temperatures, recorded at the nearest meteorological station (Begumpet).

due regard to environmental temperature. Tissues were plated in agar and doubtful colonies checked by mouse inoculation.

With guinea-pig carcases at 82° F. the organisms survived in the spleen and bone marrow for less than two days. In similar carcases at 52° F. they survived in the spleen and heart blood for over six days and in the bone marrow for between eight and 12 days.

Carcases of goats dead of anthrax were left lying in a shed at Mukteswar during June and July, at air temperatures ranging from 64° to 74° F. Results were as follows :—

Bone marrow (femur).	12 goats.
Positive on days	3, 5, 6, 7, 7, 7, 8.
Negative „	6, 6, 7, 7, 7, 9, 9, 10.
Spleen. Ten goats.	
Positive on days	2, 3½, 4.
Negative „	3, 4, 4, 5, 5, 5, 5½.
Heart blood. Nine goats.	
Positive on days	3, 3, 4,
Negative „	3, 4, 4½, 5½, 6.

In a carcase stored at 50° to 60° F. (October at Mukteswar), bone marrow was positive for anthrax for 24 days after death. Wulff

(1912) and Grabert (1915), in Europe, have noted still longer periods of survival in the case of bone marrow.

Pfeiler and Neumann (1912) have pointed out that anthrax bacilli may survive in the vessels of the skin longer than in other tissues, *e.g.*, in sheep for eleven to 43 days. This point was examined in the case of goat carcases, other than those above, kept in air at 50° to 60° F. With five such goats, anthrax bacilli survived for days : >5, 7, 12, 16, >11 and <21.

DISCUSSION

In connection with the epizootiology of anthrax under conditions like those of India, at least three important points, touched on in this work, emerge : (1) the relatively long survival of the bacillus in the bone marrow and skin of animals dead of anthrax ; (2) survival of the bacillus in dry soil ; (3) the period of time necessary for the bacillus to form spores.

The first point needs no further comment. The prolonged survival of the bacillus in dry *sterile* soil under laboratory conditions may have some practical importance. This, however, is conjectural, partly because moisture and solar effects, which may act as opposing influences, are liable to intervene. Nevertheless, one can imagine conditions in practice where anthrax blood is shed on light or sandy dry soil in shaded localities or where bacilli are sheltered from the sterilising influence of the sun by superficial layers of soil. When sooner or later temperature and moisture conditions become favourable, sporulation might occur. The third point, rate of sporulation in nature, is important, partly again in view of possible intervention by opposing forces, in this instance desiccation or putrefaction. As regards putrefaction, the action of certain putrefactive bacteria in " antagonising " anthrax bacilli has long been known and the practical significance of this fact is realised particularly in the matter of diagnosis. The importance of putrefaction in the epizootiology of the disease has probably not been sufficiently emphasised. Again, although the temperature of the water in streams and pools is often very favourable for sporulation, competition by saprophytic bacteria is a possibility to be considered.

CONCLUSIONS

1. Under favourable temperature (98° to 90° F.) and other conditions, sporulation of the anthrax bacillus, whether in a dilute serum medium or blood, has commenced by eight to ten hours ; at less favourable temperatures (70° F.) by 24 hours.

Sporulation on the opened carcase is largely dependent on the surrounding air temperature. At 90° F. spores are formed in the blood exuding from severed neck vessels, whereas at 60° to 70° F. the bacilli gradually disintegrate with the growth of contaminants. In blood removed from the carcase and stored at this lower tempera-

ture but at the same time protected from gross contamination, sporulation may proceed slowly.

Sporulation is possible in dry sterilised soil to which water is added to give a moisture content of about 3 per cent.

Spores in bottles of sterile muddy water, stored in a pond on the plains of North India, survived in undiminished numbers during an experiment lasting just over two years.

2. Survival of anthrax bacilli in blood dried on glass slides depends on the rate of drying and the conditions of storage. Survival is promoted by slow drying in relatively moist air at room temperature followed by storage in dry air at room temperature. Survival for 60 and 90 days was noted under these circumstances. The converse conditions bring about comparatively rapid death of the organism.

A few bacilli survived as long as 100 days in anthrax blood dried in tubes and exposed in the winter on the plains of North India to the daily fluctuating temperature and humidity.

Of surfaces tested, the following were bactericidal for anthrax bacilli in blood: iron, zinc, copper, brass, limewash, lead paint and tar.

The bacilli survived for over 634 days in blood mixed with dry sterile soil and stored in dry air at room temperature; in wet soil at air temperatures of 60° to 70° F. they survived for 20 days in unsterilised and 38 days in sterilised soil.

In goats dead of anthrax the bacilli commonly survive for a week in bone marrow (air temperature 64° to 74° F.) and for about two weeks in the skin (air temperature 50° to 60° F.). Survival in these situations is mainly a question of air temperature, and much longer periods have been noted by other workers.

3. Records are given of the mean temperatures of the water in ponds in North and South India over one year.

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REFERENCES

Bongert, J. (1904*a*). *Zbl. Bakt. Orig.*, **35,** 14; (1904*b*). *Ibid.*, 168.
Buxton, P. A., and Mellanby, K. (1934). *Bull. ent. Res.*, **25,** 171.
Grabert, K. (1915). *Z. InfektKr. Haustiere*, **16,** 324.
Heim, L. (1905). *Z. Hyg. InfektKr.*, **50,** 123.
Jacobsthal, E., and Pfersdorff, F. (1901). *Z. InfektKr. Haustiere*, **1,** 102.
Koch, R. (1876). In Cohn, F. *Beitrage zur Biologie der Pflanzen*, **2,** 277.
Mennucci, G. (1934). *Biol. Abstr.*, **8,** 131.
Migula, W. (1897). *System der Bakterien* (*Allg. Teil*), **1,** 173.
Minett, F. C., and Dhanda, M. R. (1941). *Ind. J. vet. Sci. anim. Husb.*, **11** 308.

Momont, M. (1892). *Ann. Inst. Past.*, **6,** 21.
Pfeiler, W., and Neumann, K. (1912). *Archiv wiss. prakt. Tierheilk.*, **38,** 266.
Preisz, H. (1904). *Zbl. Bakt. Orig.*, **35,** 416.
Rahm, O. (1945). *Bact. Rev.*, **9,** 1.
Schipp, C. (1906). *Zbl. Bakt. Ref.*, **38,** 744.
Sirena, S. (1906). *Ibid.*, **38,** 406.
Stephanidis, P. (1899). *Archiv Hyg., Berl.*, **35,** 1.
Watts, P. S. (1945). *J. Path. Bact.*, **57,** 191.
Weil, R. (1899). *Archiv Hyg., Berl.*, **35,** 355.
Wulff, F. (1912). *Z. InfektKr. Haustiere*, **12,** 266.

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LEAD AS A NUTRITIONAL HAZARD TO FARM LIVESTOCK

III. Factors Influencing the Distribution of Lead in the Tissues

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INTRODUCTION

In the previous paper (Blaxter, 1950) it was shown that although lead was only slightly absorbed from the digestive tract of sheep and rabbits, a very large part of the lead which was absorbed was retained in the body. The present paper is concerned with the internal transport and deposition of this absorbed lead.

THE REMOVAL OF LEAD FROM THE BLOOD STREAM OF THE SHEEP

Table I shows the lead content of the blood of sheep E following the injection of 400 mg. of lead as the acetate. Fig. 1 shows the cell and serum concentration of lead plotted separately, the data

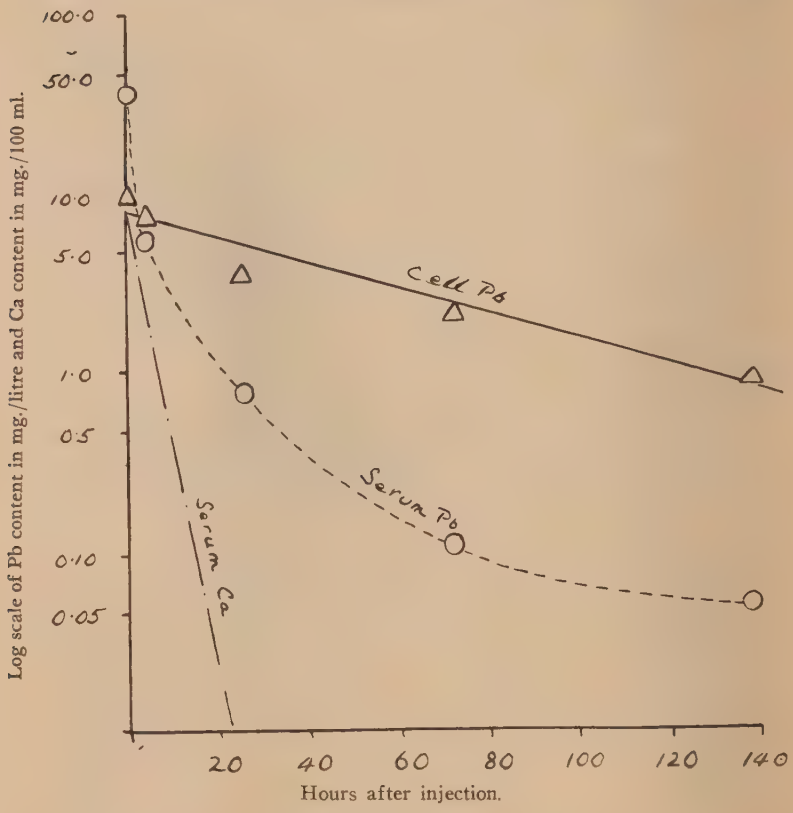
Table I

Blood Lead following Intravenous Injection of Sheep E with 400 mg. Pb as the Acetate in Normal Saline

Hours from injection	*Blood Pb mg. per l.*	*Hours from injection*	*Blood Pb mg. per l.*
108 before	0·20	0·08 after	72·8
60 „	0·09	3 „	40·5
12 „	0·07	12 „	13·5
0·1 „	0·08	24 „	4·8
		54 „	2·3
		78 „	2·4
		114 „	1·1
		282 „	1·0

being determined from cell volume, serum lead concentration and whole blood concentration simultaneously determined. Separate determinations on cells and plasma, involving the use of oxalate as anti-coagulant, gave variable results, presumably due to co-precipitation of lead oxalate in the more concentrated samples. From the table it will be noted that the whole blood Pb concentration rose to 7·3 mg. per 100 ml. immediately following the injection, a value which is in agreement with expectation on the basis of calculations of the blood volume of the sheep. Subsequently the value fell, but even after 12 days the concentration was still ten times the normal, pre-injection value.

FIG. 1.—The removal of injected lead from the serum and cells of sheep blood plotted on arithlog co-ordinates and a comparison with the rate of removal of injected calcium.



Key

Vertical axis = Log scale of Pb content in mg. per l., and Ca content in mg. per 100 ml.
Horizontal axis = Hours after injection.

On plotting these data it was found that the fall in total blood lead concentration did not obey the relationship normally found on the injection of simple salts into the blood stream. Normally, the equation relating blood concentration x, to time, t. is $X = A\,e^{-kt}$ where A is the initial concentration and k is a constant. In this case the rate of fall in concentration, $\frac{dX}{dt}$ is proportional to the amount of the constituent present, X. The cell lead determinations shown in Fig. 1, however, show a far better fit to this exponential rate. Even so, the serum lead curve is definitely not of this type, markedly

differing in this respect from the behaviour of injected Ca in the ruminant (Allcroft, 1947).*

The very slow rate of fall of the cell lead concentration suggests that this lead, adsorbed on the surface membrane of the corpuscles (Cantarow and Trumper, 1944), is only released very slowly to the circulation. The protracted fall of the serum lead curve is probably a reflection of this slow release of Pb ions from the cells rather than the limited capacity of the tissues to take up lead from the circulation. The very slow fall in whole blood lead concentration in the sheep differs markedly from the behaviour of colloidal lead phosphate injected intravenously into the horse where none can be demonstrated at the second hour following injection (Newman, 1930).

To study further the cell-serum relationship, small quantities of lead nitrate were added to defibrinated cow blood *in vitro*. It was found that the cells accounted for 63 to 70 per cent. of the total blood lead, even when the latter was increased to 31 mg. per l. A larger proportion—85 to 90 per cent.—was found bound to the cells in normal blood and in blood taken from sheep which had been given lead in their diet, and no reason for the discrepancy could be found. Behrens and Pachur (1927) have found, however, that there are species differences in the affinity of red blood corpuscles for lead, so that the difference between the *in vitro* studies using cow blood and the *in vivo* studies using sheep may reflect a species difference. The salient point remains, namely, that the cells account for the larger part of the total blood lead in both species.

In the experiment with sheep E, the lead concentration in the serum was far higher than the lead concentration in the urine for the whole experiment, suggesting that only a small part is able to pass the glomerulus of the kidney. An attempt was therefore made to ascertain how much of the plasma lead would pass through a collodion membrane. Lead was therefore added to serum *in vitro*, a determination made, and lead determined on the protein-free ultra-filtrates. When the Pb concentration was increased to 10 mg. per l., far less than 1 per cent. of the lead could be found in the ultra-filtrate and with smaller concentrations very similar results were obtained. When the serum Pb was raised to 2 mg. per l., even the analysis of 50 ml. of ultra-filtrate failed to reveal significant amounts of lead. Similar conclusions were reached on the basis of the analysis of posterior chamber fluid from the eyes of rabbits given large quantities of lead. Even when serum concentrations were very high, no lead could be detected in this protein-free fluid. Lead has been found in cerebrospinal fluid in man (Schmitt and

* The equation relating the rate of fall of serum Ca following an injection of Ca is $\frac{dX}{dt} = -K(X-B)$, where B is the level of Ca in the serum prior to injection. In the case of Pb, which is only present in the blood in minute amounts, the value of B in the comparable equation is zero.

Basse, 1937), the concentration bearing, however, little relation to the concentration of lead in the blood.

From these experiments it can be concluded that Pb which enters the circulation of the sheep is largely bound by the cells, while the serum lead is not freely diffusable, suggesting that it is largely bound to protein. It can be postulated, then, that in the dissociation equation (c.f. McClean and Hastings, 1935) $\frac{(Pb^{++})\ (Protein^{-})}{(Pb\ proteinate)} = K$, the value of K is very small. This entails that when lead is introduced into the circulation in large quantities the percentage which is ionised will be very small, and when small quantities are introduced the percentage will be much greater. Presumably, precipitation of the serum proteins will occur at very high concentrations of Pb.

THE DISTRIBUTION OF LEAD IN THE TISSUES OF RABBITS FOLLOWING ORAL AND INTRAVENOUS ADMINISTRATION

The Recovery of Pb from Rabbit Tissues

Before discussing the experiments in which various quantities of lead were given to rabbits, it is of interest to compute the recovery

TABLE II

RECOVERY OF LEAD FROM RABBITS INJECTED INTRAVENOUSLY WITH 20 MG. AND 30 MG. OF PB AS THE ACETATE FOUR DAYS PRIOR TO SLAUGHTER

Tissue	*Rabbit given 20 mg. Pb*			*Rabbit given 30 mg. Pb*		
	Weight of tissue g.	*Pb content mg./kg.*	*Total Pb in mg.*	*Weight of tissue g.*	*Pb content mg./kg.*	*Total Pb in mg.*
Lung	10·7	10·08	0·108	11·4	15·56	0·177
Heart	4·8	1·12	0·005	8·1	1·86	0·015
Liver	56·2	75·5	4·243	68·9	145·2	10·004
Kidney	14·8	13·76	0·204	19·2	21·08	0·405
Spleen	1·1	107·5	0·115	1·7	132·9	0·226
Brain	8·9	0·90	0·008	10·2	1·19	0·102
Pancreas	1·0	4·06	0·004	1·5	3·86	0·006
Colon	17·1	2·05	0·035	14·4	2·56	0·037
Small intestine ...	31·3	1·81	0·056	37·2	2·16	0·080
Rectum	10·2	2·05	0·021	8·2	2·80	0·023
Bone (1)	348·0	13·5 *	4·698	454·4	13·6 *	6·181
Marrow (2) ...	46·1	199·0	9·154	62·0	227·7	14·117
Blood (3)	187·0	1·45	0·271	245·0	3·9	0·955
Muscle (4)	937·0	0·70	0·655	1,229·0	0·90	1·106
Total	—	—	19·571	—	—	33·344

(1) 14·8 per cent. of body weight
(2) 2·2 per cent. of body weight
(3) 8 per cent. of body weight
(4) 40 per cent. of body weight

* Estimated as a difference—injected minus control concentration.

Muscle, bone, blood and bone marrow were not weighed and estimations made based on observations by Shohl (1939) and Deitz (1944).

of lead from the bodies of two rabbits which were injected with lead. Table II shows that when 20 mg. were injected 19·6 mg. were recovered four days later; when 30 mg. were injected, 33·3 mg. The excretion of the lead which occurs during the period of four days between injection and slaughter entails some loss, while the small positive error in the case of the second rabbit is undoubtedly accounted for by errors in the estimation of the weights of marrow, bone and muscle from formulae (Shohl, 1939; Deitz, 1944). Comparable data for rabbits given single doses of lead by mouth are shown in Table III. It will be seen that 9·9 mg. were recovered

TABLE III

RECOVERY OF LEAD FROM RABBITS POISONED ORALLY WITH 1 G. AND 2 G. OF PB RESPECTIVELY

Tissue	*Rabbit, 1 g. Pb*			*Rabbit, 2 g. Pb*		
	Weight of tissue in g.	*Pb content in mg./kg.*	*Total Pb in mg.*	*Weight of tissue in g.*	*Pb content in mg./kg.*	*Total Pb in mg.*
Lung	15·5	2·64	0·041	14·3	4·30	0·061
Heart	7·2	0·60	0·004	6·0	0·80	0·005
Liver	68·5	19·14	1·311	74·6	54·62	4·075
Kidney	16·9	29·85	0·504	18·0	43·63	0·785
Spleen	1·5	17·71	0·027	1·8	25·20	0·045
Brain	10·5	0·53	0·006	10·8	0·89	0·009
Pancreas	0·6	39·00	0·023	2·6	5·62	0·014
Colon	17·7	21·36	0·041	18·5	11·76	0·215
Small intestine ...	37·8	7·14	0·269	31·2	7·30	0·227
Rectum	11·5	2·28	0·026	11·1	9·11	0·101
Marrow	62·3	4·19	0·261	61·4	6·73	0·413
Muscle	1,132·0	0·10	0·113	1,115·6	0·35	0·390
Bone	418·8	16·7	6·994	412·8	27·7	11·434
Blood	198·1	1·72	0·341	195·2	1·75	0·342
Total	2024	—	9·911	2001	—	18·116
Residual ...	807	—	—	788	—	—
Weight of rabbit ...	2831	—	—	2789	—	—

For footnotes, see Table II

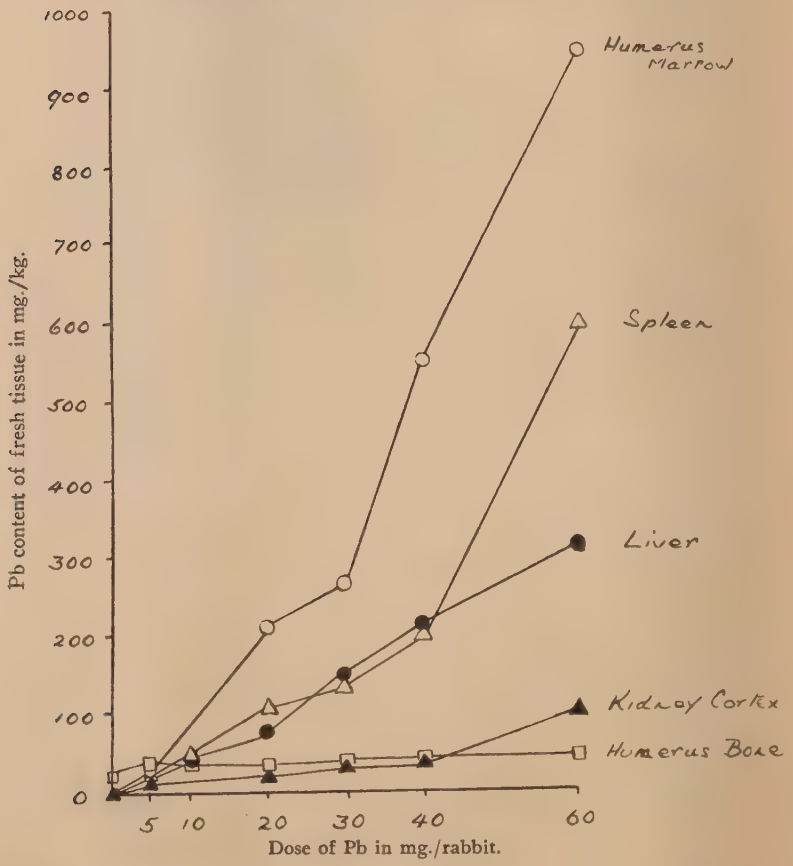
from the rabbit given a single dose of 1 g. and 18·1 mg. from the rabbit given 2 g. These recovery data in which lead has been absorbed from the digestive tract are in excellent agreement with the absorption experiments of the previous paper (Blaxter, 1950).

Lead Deposition in the Tissues of Intravenously Poisoned Rabbits

In the first part of Table IV tissue analyses are shown for rabbits given increasing quantities of lead by intravenous injection. Part of the data is shown in Fig. 2. The most striking feature of these results is the enormous increase in the lead content of the spleen and bone marrow, compared with the slower increase in the lead content of the kidney and compact bone. The increase in the

lead content of the whole bone was largely accounted for by the high lead content of the marrow, the difference between the femur and the humerus reflecting the different percentage of marrow in these two bones. The spleen lead concentration was not in direct relationship with dose for a considerable splenic hypertrophy occurred and the spleen weight in mg. per kg. of body weight rose from 420 to 920. This was accompanied by a fall in spleen dry matter from 44·5 per cent. to 25·1 per cent. in extreme cases. Such a splenic hypertrophy has been noticed by Laug and Morris (1938) in experimental lead poisoning. Slight liver and kidney hypertrophy

FIG. 2.—The tissue concentration of Pb in intravenously poisoned rabbits.



Key

Vertical axis = Pb content of fresh tissue in mg. per kg.
Horizontal axis = Dose of Pb in mg. per rabbit.

TABLE IV

LEAD CONTENT OF RABBIT TISSUES IN MG. PER KG. FRESH TISSUE FOUR DAYS AFTER INJECTION OR DOSAGE

Tissue, etc.	*Rabbits poisoned by I.V. injection*							*Rabbits poisoned by mouth*		
Rabbit No.	1	2	3	4	5	6	7	8	9	10
Wt. rabbit, kg.	2·54	2·38	2·05	2·34	3·07	2·57	2·43	2·83	2·79	2·57
Dosage mg.	0	5	10	20	30	40	60	1,000	2,000	4,000
Dosage mg. per kg.	0·00	2·10	4·88	6·41	9·77	15·56	24·69	353	707	1,556
Lung ...	0·00	1·46	3·30	10·1	15·6	26·9	67·0	2·66	4·30	5·37
Heart muscle	0·06	0·80	1·30	1·12	1·86	2·27	3·54	0·60	0·80	1·55
Liver ...	0·40	14·3	42·6	75·5	145·2	211·7	316·0	19·1	54·6	137·4
Kidney—										
Whole ...	0·20	9·69	9·20	13·8	21·1	27·6	61·2	29·9	43·6	299·0
Cortex ...	0·95	13·9	—	18·9	37·1	38·4	105·8	40·2	62·6	370·6
Medulla	0·00	6·66	—	5·84	5·65	8·04	13·4	12·5	28·7	161·7
Spleen ...	0·10	16·9	40·8	107·5	132·9	204·5	604·2	17·7	25·2	62·9
Brain ...	0·00	0·55	0·60	0·90	1·19	1·34	1·40	0·53	0·89	1·22
Pancreas	0·00	4·86	3·70	4·06	3·86	6·53	4·38	39·0	5·62	7·31
Colon ...	0·00	1·28	1·40	2·05	2·56	3·76	4·10	2·36	11·8	40·11
Small intestine ...	1·00	1·08	2·40	1·81	2·16	3·41	2·83	7·14	7·30	9·73
Rectum ...	0·10	1·32	0·40	2·05	2·80	2·30	2·27	2·28	9·11	16·4
Muscle ...	0·00	0·53	0·70	0·55	0·70	0·82	1·03	0·10	0·35	0·56
Blood ...	0·30	1·19	2·50	1·45	3·90	4·54	1·75	1·72	1·75	3·08
Femur—										
Whole	12·9	25·9	47·9	104·8	114·8	219·7	265·6	35·0	36·9	41·5
Bone ...	15·2	32·3	—	25·5	28·7	40·5	42·0	33·8	42·1	48·3
Marrow	0·00	14·3	—	188·3	227·7	584·2	964·1	3·62	6·72	12·5
Humerus—										
Whole	12·5	32·0	57·2	112·8	113·0	231·6	399·0	35·2	38·4	42·4
Bone ...	18·0	34·3	—	32·2	31·6	40·5	41·9	32·9	46·5	50·6
Marrow	0·00	13·8	—	211·2	266·2	554·2	951·5	4·19	6·73	13·1
Urine (bladder)	—	—	—	—	1·57	3·54	13·61	3·15	7·77	* —
Bile ...	—	—	—	—	65·5	53·4	28·8	13·5	3·5	329·6

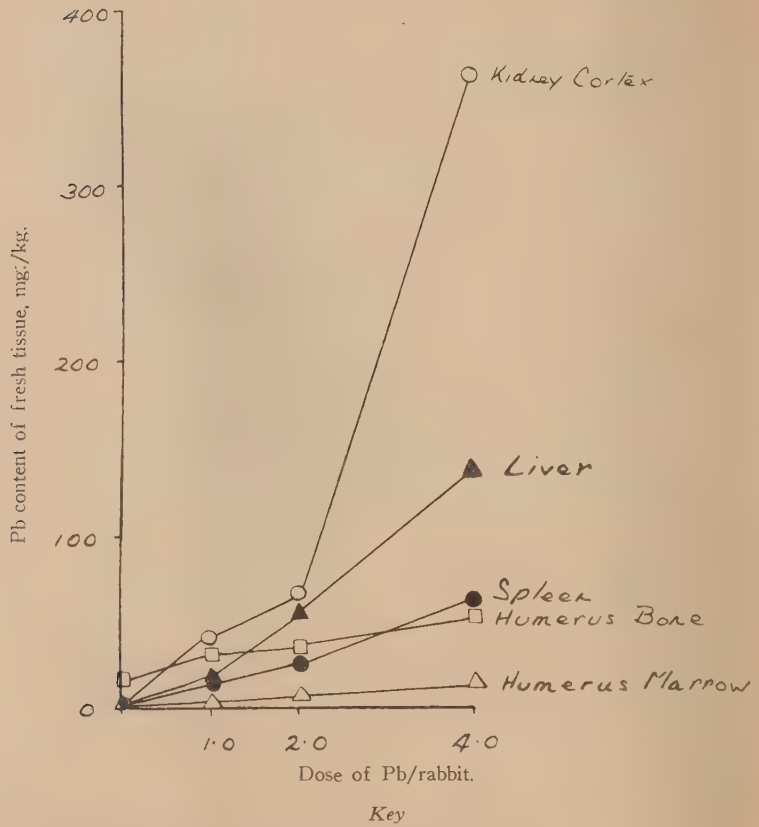
* Unfortunately none in bladder.

was also noted but the variation among individual rabbits precluded any conclusion. In the case of the kidney, in agreement with the results obtained by Calvery, Laug and Morris (1938), the medulla lead concentration was far lower than that of the cortex.

Lead Deposition of the Tissues of Rabbits given Lead by Mouth

In the second part of Table IV and in Fig. 3, comparable data are shown for rabbits given single doses of lead by mouth. On the basis of the data on the absorption of lead from the digestive tract previously presented, these rabbits absorbed approximately 10, 20 and 40 mg. of lead respectively, and are thus comparable to the

FIG. 3.—The tissue concentration of Pb in orally poisoned rabbits.



Key

Vertical axis = Pb content of fresh tissue in mg. per kg.
Horizontal axis = Dose of Pb in g. per rabbit.

corresponding rabbits given similar quantities by vein. In these orally poisoned animals, the lead concentration in the spleen and bone marrow did not increase to the extent which had been observed in the animals given lead intravenously. Kidney lead and bone lead increased very considerably. Thus, in comparable animals receiving either 4 g. of lead by mouth or 40 mg. of lead by vein, the lead content of the humerus marrow was 40 times greater in the intravenously dosed animal, while the lead content of the kidney cortex was ten times greater in the rabbit given lead by mouth. In every case the lead content of the liver of the animal given lead by vein was approximately double the lead content of the liver of the animal absorbing comparable amounts of lead from the digestive tract. A further difference was in the lead content of the compact

bone. In each comparison the animal absorbing lead from the digestive tract had the higher concentration of lead in the compact bone.

The Differential Distribution of Lead in the Tissues

It is obvious that there is a markedly different distribution of lead in the tissues of rabbits in which comparable amounts of lead have entered the body by different routes. That the same is true of the sheep can be seen from Table III of the previous paper (Blaxter, 1950), for in the case of the sheep injected with lead the content of the liver was higher than that of the kidney, and in the case of the sheep given lead by mouth the reverse was true.

It appears that lead entering the systemic circulation tends to invade the reticulo-endothelial system, represented by the lung, the spleen, the bone marrow and the liver, while lead entering the body through the gut wall invades the bone and the kidney. This differential distribution is shown graphically in Fig. 4, where it can be seen that 48 per cent. of the injected lead entered the reticulo-endothelial system, while only 3 per cent. of the lead absorbed from the gut was taken up ; and that while 24 per cent. of the injected lead was found in the compact bone, 63 per cent. of the absorbed lead was present there.

An attempt was made to stain the lead present in sections of spleen, bone, marrow and kidney using Okkel's stain (Romeis, 1932). This stain consists of a picric acid solution containing 3 ml. w.v. nitric acid saturated with H_2S. This stained the kidney lead but not the lead in the spleen or bone marrow of rabbits poisoned by intravenous injection. Increasing the concentration of nitric acid caused a little blackening of the spleen and bone marrow lead. This attempt to stain the lead present in the reticulo-endothelial tissues suggests that the lead was held there in a different manner from the lead deposited in the kidney.

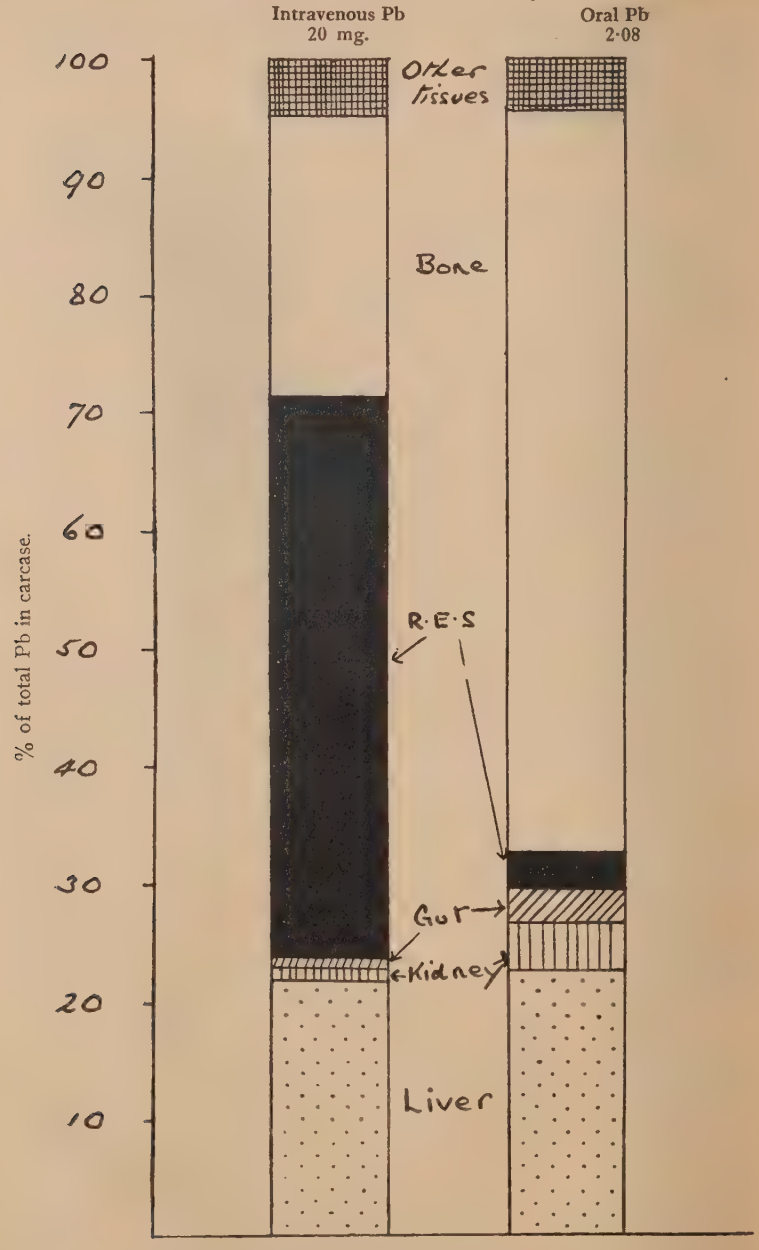
The ability of bone to take up these relatively enormous amounts of lead given by mouth can be assessed from the data shown in Table IV. Lead deposition in the bones has been arrayed against dose and is shown in Table V. It can be seen from this table

Table V

Uptake of Lead by Bone and Kidney in Oral Poisoning

Amount of lead given		*Lead content of bone (Femur)*		*Lead content of kidney*
Actual g.	*mg. per kg.*	*Actual*	*Corrected for control*	
0·0	—	15·2	0·0	0·20
1·0	353	33·8	18·6	29·9
2·0	707	42·1	26·9	43·6
4·0	1,556	48·3	33·1	299·0

FIG. 4.—The distribution of tissue lead in rabbits retaining comparable amounts of lead administered in different ways.



Key

1st column heading = Intravenous Pb 20 mg.
2nd column heading = Oral Pb 2·0 g.
Vertical axis = Per cent. of total lead in carcass.

that the increase in the lead content of bone with increasing oral intake was not one of direct proportionality after deduction of the lead content of the control bone. It would appear that bone was able to take up lead rapidly when the dose was small, but with higher doses the bone could not deal with the excess and whilst more lead was deposited it was not in direct proportion to the dose. This probably accounts for the enormous content of lead in the kidneys of the last rabbit in the series and for the very severe damage found in the kidneys of rabbits receiving comparable doses. The kidney evidently tries to deal with that lead which cannot be stored in the bones in these orally poisoned animals.

The Rate of Removal of Lead from the Tissues

To determine the rate at which lead was removed from the body following a single exposure, rabbits were injected with 10 mg. of lead and killed at varying lengths of time. The results are shown in Table VI. It will be noted that the rate of decline in the lead con-

TABLE VI

LEAD CONTENT IN MG. PER KG. OF TISSUES OF RABBITS KILLED AT INTERVALS FOLLOWING THE INTRAVENOUS INJECTION OF 10 MG. Pb

Tissue	*Normal*	*Number of days following injection*					
	0	2	4	8	16	32	59
Liver	0·4	28·0	42·6	21·7	8·6	16·1	4·4
Spleen	0·1	60·1	40·8	30·8	18·0	6·1	2·4
Kidney	0·2	10·5	9·2	6·2	2·7	6·5	2·0
Humerus	12·5	54·8	57·2	44·5	27·5	21·2	17·2
Femur	12·9	31·7	47·9	37·2	25·1	19·9	16·9
Lung	0·0	2·6	3·3	2·4	0·7	0·3	0·6
Heart	0·1	1·3	1·3	1·6	0·2	0·0	0·3
Brain	0·0	1·3	0·6	0·2	0·4	0·0	0·2
Small intestine	1·0	2·8	2·4	1·8	0·4	1·1	0·2
Colon	1·0	1·8	1·4	0·8	0·5	0·4	0·1
Rectum	0·1	3·0	0·4	0·7	0·3	1·0	0·4
Muscle	0·0	0·9	0·7	0·1	0·8	0·2	0·0
Blood	0·3	3·4	2·5	0·5	0·6	0·6	0·4

tent of the liver, the spleen and the bones (whole bones including marrow) were comparable to one another, but the lead content of the kidney declined much more slowly. This suggests that lead deposited in the reticulo-endothelial system is comparatively easily removed. Bone and bone marrow were analysed separately in the rabbit killed 59 days after the injection and the concentrations compared with those found in the control rabbit. There was little difference in the content of the compact bone, but lead was still present in the marrow. Calculations from the data in Table VI indicate that some 5 to 10 per cent. of the injected lead was still present in the tissues even after 59 days following the injection.

This emphasises the tenacity of the tissues in retaining lead which has gained access to the body.

DISCUSSION

The differential distribution of lead in the tissues following the two methods of administration is of some interest. The localisation of injected lead acetate parallels the distribution of reticulo-endothelial cells, thus appearing to be quite comparable to the distribution of injected colloidal lead phosphate (Dilling and Haworth, 1929; Cantarow and Trumper, 1944), while the same quantity of lead absorbed from the acetate given by mouth has an entirely different distribution. The explanation undoubtedly lies in the relative amounts of lead reaching the circulation at any one time. The local concentration of lead injected intravenously must be sufficient to cause a partial precipitation of the plasma proteins and these would then be filtered off by the reticulo-endothelial system. In the case of orally administered lead, absorption is spaced over several hours and, as shown by the blood studies above, the percentage of the total lead which would be bound to protein following absorption from the tract would be much smaller and never at the point at which precipitation would occur. Even when 4 g. of lead were given by mouth, roughly equivalent to 40 mg. by vein, the amount of lead found in the bone marrow was only equivalent to that found on the injection of 5 mg. of lead.

This differential distribution casts some doubt on the validity of the assumptions made in the previous paper, that the relative importance of the kidney and the liver in excreting absorbed lead may be measured by the injection of lead into the normal animal. Undoubtedly, this method can only be used where the element under consideration is completely ionised, or where by continuous injection the blood level of the element is kept at relatively low levels. In the present instance it would appear that a considerable part of the lead injected into the circulation is effectively removed before immediate excretion can occur, held by the reticulo-endothelial cells and subsequently slowly released to the circulation.

The present studies show that for diagnostic purposes in animals which have been eating diets containing lead, analysis of the kidney cortex would give a good indication of exposure. Not only does this tissue take up lead more avidly than other tissues, but over a long period of time the concentration remains high, presumably due to the fact that lead released from other tissues in small quantities is taken up by the kidneys in an attempt to excrete it in the urine.

SUMMARY

Following the injection of lead into the vein of a sheep, it was found that the whole blood concentration dropped very slowly. The lead content of the corpuscles dropped at a slow exponential rate,

while the lead content of the serum, although dropping initially much more rapidly, also declined very slowly.

It was found that in normal sheep and in sheep given small and large quantities of lead by mouth the cells accounted for 85 to 90 per cent. of the blood lead. *In vitro* studies on cow blood indicated that the cells accounted for 63 to 70 per cent.

On the basis of ultra-filtration studies and the analysis of protein-free fluids from rabbits, it was concluded that very little of the serum lead is freely diffusable, the larger part being bound to the serum proteins.

The distribution of lead following both oral and intravenous administration was determined in the tissues of rabbits. It was found that lead when given intravenously as the acetate followed the distribution of the reticulo-endothelial cells of the body. When given by mouth, most of the lead was found in the skeleton with very high concentrations in the kidney. The carcase analyses of two sheep agree with this differential distribution.

With increasing oral dosage the amount of lead stored in the bones increased, but there was an indication that the bone cannot deal with large excesses which are then deposited and cause damage in the kidney, particularly the cortex.

After the intravenous administration of lead, there was still 5 to 10 per cent. present in the tissues 59 days after the injection.

The results are discussed in relation to the factors causing the differential distribution of lead and its implication.

REFERENCES

Allcroft, W. M. (1947). *Vet. J.*, **103,** 157.
Behrens, B., and Pachur, R. (1927). *Arch. f. exp. Pharm.*, **122,** 319.
Blaxter, K. L. (1950). *J. comp. Path.*, **60,** 140.
Calvery, H. O., Laug, E. P., and Morris, H. J. (1938). *J. Pharm.*, **64,** 364.
Cantarow, A., and Trumper, M. (1944). "*Lead Poisoning.*" Williams & Wilkins Co.: Baltimore.
Deitz, A. A. (1944). *Proc. Soc. exp. Biol. and Med.*, **57,** 60.
Dilling, W. J., and Haworth, E. F. (1929). *J. Path. Bact.*, **32,** 753.
Laug, E. P., and Morris, H. J. (1938). *J. Pharm.*, **64,** 388.
McClean, F. C., and Hastings, A. B. (1935). *J. biol. Chem.*, **108,** 285.
Newman, R. K. (1930). *Med. J. Austr.*, **1,** 170.
Romeis, B. (1932). "*Tachenbuch der Mikroskopischen Tecknik.*" B. Auflage: München und Berlin.
Schmitt, F., and Basse, W. (1937). *Klin. Wochschr.*, **16,** 65.
Shohl, A. T. (1939). "*Mineral Metabolism.*" Rheinhold Publishing Co.: New York.

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LEAD AS A NUTRITIONAL HAZARD TO FARM LIVESTOCK

IV. Distribution of Lead in the Tissues of Bovines after Ingestion of Various Lead Compounds

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INTRODUCTION

The experiments reported in the two previous papers in this series (Blaxter 1950, *a*, *b*) have largely dealt with sheep experiments using metabolism methods. The experiments reported in the present paper have been made with young cattle, using larger numbers of animals and feeding trial methods.

The literature on lead poisoning in farm animals—chiefly acute poisoning—indicates that the young calf is extremely susceptible to lead poisoning (White and Cotchin, 1948; Fenstermacher *et al.*, 1946; Shrewsbury *et al.*, 1945; Steyn, 1940), but little is known of the changes which occur in the lead content of the blood and tissues in such cases, nor is the susceptibility of the calf to chronic intoxication over long periods of time in any way established. The present paper deals with blood, tissue and faecal concentrations of lead after single lethal doses of various lead compounds and after ingestion of small quantities over long periods. The wider implications of these studies in terms of the reduction of productivity or the death of cattle due to lead ingestion under farm conditions in this country are dealt with in the subsequent paper (Allcroft and Blaxter, 1950).

EXPERIMENTAL

The experiments were carried out with young calves aged from a few days to ten months, reared under normal farm conditions, housed in concrete stalls and fed rations commensurate with their age and condition. All lead compounds were given by mouth. Samples of whole blood were taken into oxalate and, at slaughter or death, the tissues were removed from the body as quickly as possible and stored in glass jars in the refrigerator until analysed. Lead was determined by the method described in the first paper of this series (Blaxter and Allcroft, 1950).

RESULTS

The Lead Content of Blood and Tissues of Normal Animals

In order to obtain an indication of the concentration of lead in the liver and kidney of normal animals, these tissues were taken from 31 cattle slaughtered for other purposes. The animals ranged in

TABLE I

RANGE OF LEAD CONTENT OF NORMAL KIDNEY AND LIVER TISSUE FROM SHEEP AND CATTLE

Animals	Number of animals	Lead content in mg. per kg. of fresh tissue	
		Liver	Kidney
Normal sheep	5	0·6–1·2	0·3–0·8
Normal lambs	3	All less than 1·0	All less than 1·0
Normal calves	10	0·4–1·2	0·4–1·0
Normal heifers and cows ...	13	0·3–1·5	0·3–1·5
* "Swayback" lambs born in Derbyshire	5	3·7–7·6	1·0–3·0

* Derbyshire is an area where the soil content of lead is very high, and the consumption of lead by the ewes during pregnancy can be as high as 50 mg. daily. The data are not therefore strictly comparable with the other normal tissues listed but are of interest in the interpretation of some of the data in the tables which follow.

age from six months to seven years and as far as could be ascertained had never had access to abnormal quantities of lead. The results are shown in Table I. These data may be compared with those for normal human beings as summarised by Cantarow and Trumper (1944). The agreement is extremely good, for the more recent analyses given in their summary show that the lead content of normal human liver and kidney is unlikely to exceed 2 mg. per kg. In this respect Fenstermacher *et al.* (1946) in studies of lead poisoning in cattle have regarded liver values of up to 3 mg. per kg. as within the normal range.

A similar study was made of the levels of lead in the whole blood of young calves. The figures are shown in Table II. A comparison with data for man (Cantarow and Trumper, 1944) indicates that these results are lower than those encountered in adult human beings, for the upper limit of the normal lead content of whole blood in the latter is given as 0·8 mg. per l. The results for these young calves are, however, in substantial agreement with the results for normal sheep and goats, as shown in the table, and are in agreement

TABLE II

LEAD CONTENT OF BLOOD OF NORMAL FARM ANIMALS OF DIFFERENT SPECIES

Animal	*Number of samples*	*Mean Pb content in mg. per l.*	*Standard deviation*
Calf	30	0·129 ± 0·010	± 0·0561
Goat	4	0·130 ± 0·026	± 0·0316
Sheep	12	0·139 ± 0·010	± 0·0348
Horses	2	0·140 ± 0·010	—

TABLE III

INCREASE IN CONCENTRATION OF LEAD IN WHOLE BLOOD AFTER A SINGLE ORAL DOSE OF VARIOUS LEAD COMPOUNDS

Calf No.	Age	Amount and form of lead given: Total	Amount and form of lead given: g. kg. body weight	Result	Blood Pb in mg. per l. days after dosage: hours 0	4	1	2	3	4	5	6	7	14	21	28
862	14 weeks	31·4 g. as wet paint	0·25	Died 5th day	0·50*	1·18	1·90	1·38	1·80	—	2·34					
869	12 „	15·7 g. as wet paint	0·12	Survived	0·15	2·12	2·46	2·18	1·36	—	0·84	1·0	1·04	0·74	0·72	0·76
881	4 „	13·6 g. as dry paint flakes ...	0·23	Died 5th day	0·14	2·10	1·64	—	1·90	—	—					
865	10 „	31·5 g. as basic carbonate ...	0·24	Died 4th day	0·15	2·34	2·54	2·48	2·52	—						
891	7 „	15·7 g. as basic carbonate ...	0·22	Died 7th day	0·78*	1·58	1·70	1·94	2·05	—	2·32	2·18	1·92			
922	7½ weeks	24 g. as red lead (Pb_3O_4) ...	0·25	Died 6th day	1·16*	1·88	2·06	—	2·64	2·36	2·08	2·16				
926	5½ „	7·3 g. as red lead (Pb_3O_4) ...	0·12	Survived	1·16*	1·82	1·18	—	1·82	1·72	1·74	1·52	1·52	1·14	1·00	
976	6 „	15·5 g. as phosphate $Pb_3(PO_4)_2$	0·25	Died 4th day	1·80*	2·0	3·09	3·00	2·54	—						
951	10 „	55 g. as phosphate $Pb_3(PO_4)_2$	0·5	Died 5th day	0·12	0·90	1·66	1·64	1·34	—	—					
961	7½ „	15·9 g. as finely ground galena	0·25	Survived	0·08	0·10	0·30	0·42	—	0·42	—	0·42	—	0·38		
960	7½ „	46·6 g. as finely ground galena	0·5	Survived	0·09	0·09	0·60	0·78	—	1·04	—	0·90	—	0·96	1·02	0·66
857	8 months	80 g. as acetate in solution ...	0·52	Survived	0·84*	3·94	1·70	1·70	1·70	—	1·24	—	1·00	1·00	—	0·78
940	5 days	18 g. as acetate in solution ...	0·5	Survived	0·22	—	2·22	1·60	—	1·66	—	1·58	—	1·10 (12th day)		
891	12 „	13·6 g. as acetate in solution ...	0·35	Survived	0·15	2·26	1·36	—	1·24	—	—	1·12	—	0·95	—	0·86
941	2 „	10·5 g. as acetate in solution ...	0·35	Died 12th day	0·22	—	2·76	2·34	—	1·62	—	1·42	1·36 (10th day)			
935	9 „	9·4 g. as acetate in solution ...	0·25	Survived	0·08	2·34	1·34	1·04	1·16	1·12	0·78	—	0·76			
936	8 „	6 g. as acetate in solution ...	0·2	Survived	0·08	1·52	1·22	1·04	0·90	1·06	0·72	—	0·72			
1039	4 months	100 g. as the metal (shot) ...	0·5	Survived	0·13	—	0·22	0·20	—	0·23	—	—	0·23	0·15	0·17	
1060	4 „	70 g. as the metal (fine dust) ...	0·5	Survived	0·13	—	0·33	0·50	—	0·54	—	—	0·47	0·39	0·33	

* These high initial levels were due to pre-experimental ingestion of lead compounds.

with results obtained on normal rabbits (Blaxter, 1950*b*). On the basis of the variation of the blood lead content of these animals it has been calculated that the probability of a normal animal showing a blood lead value as high as 0·8 mg. per l. is minute, suggesting that the use of the human value of 0·8 mg. per l. as a normal upper limit for blood lead concentration in cattle and sheep is much too high for diagnostic purposes.

The Effect of Lead Ingestion on the Concentration of Lead in Blood, Tissues and Faeces. Experiments with large quantities of Lead, Dosage 0·1 to 0·5 g. per kg. body weight

Blood. The results of experiments in which relatively large quantities of lead were given to calves are shown in Table III. In Fig. 1, typical results are shown from cases of poisoning from lead paint ingestion, and in Fig. 2 similar results obtained after dosing with lead acetate. From the data in Table III and Figs. 1 and 2, it is clear that after ingestion of lead compounds there is a consider-

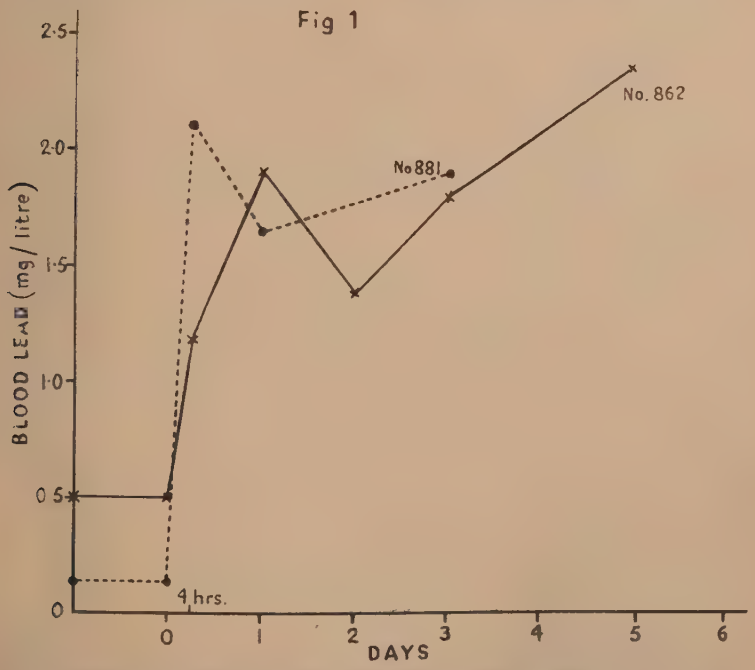

FIG. 1.—Blood lead values for two calves after a single dose of lead as paint. ● – – – – ● calf No. 881, aged four weeks, given 13·6 g. Pb as dry paint flakes, *i.e.*, 0·23 g. Pb per kg. body weight. X————X calf No. 862, aged 3½ months, given 31·4 g. Pb as wet paint, *i.e.*, 0·25 g. Pb per kg. body weight. (The high initial blood value in this animal was due to the subject having been used in a previous lead experiment.)

able and rapid rise in the amount of lead in the blood. The high value falls off rapidly at first, but instead of returning to normal within a few days, the level remains elevated for many weeks. This protracted fall is well illustrated in Fig. 3. In this case (No. 869) in which the calf survived, the blood lead was still above normal six

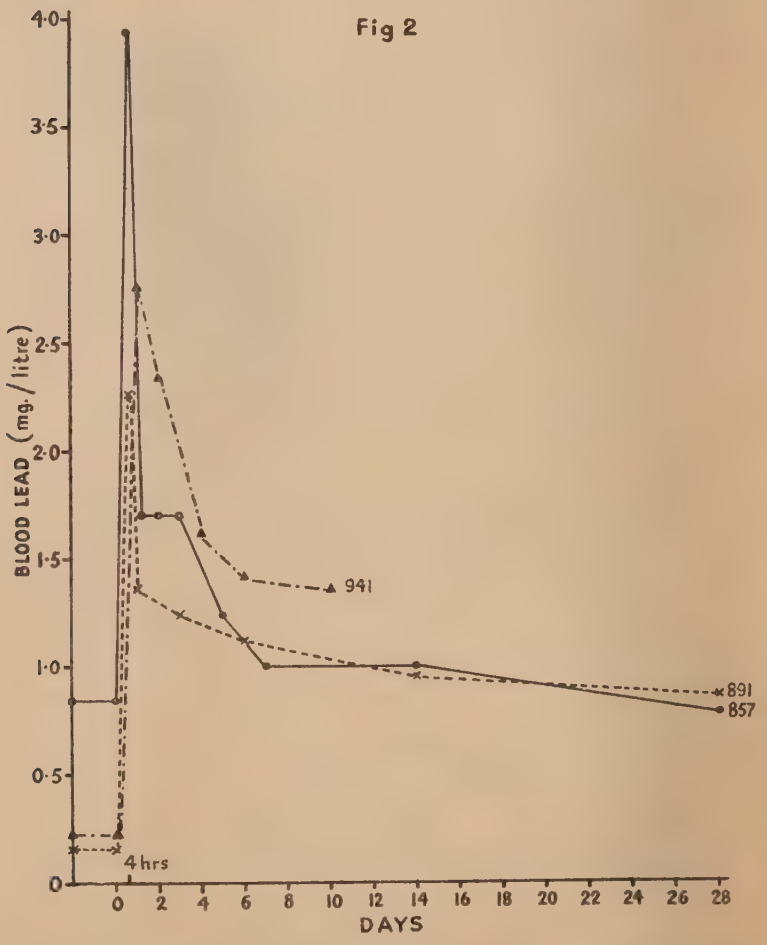

Fig. 2.—Blood lead values for three calves after a single dose of lead as acetate. ●———● calf No. 857, aged eight months, given 80 g. Pb as acetate, *i.e.*, 0·52 g. Pb per kg. body weight. Survived. X– – – –X calf No. 891, aged 12 days, given 13·6 g. Pb as acetate, *i.e.*, 0·35 g. Pb per kg. body weight. Survived. ▲— - — - ▲ calf No. 941, aged two days, given 10·5 g. Pb as acetate, *i.e.*, 0·35 g. Pb per kg. body weight. Died 12th day.

Note.—The high initial blood value in the case of calf No. 857 was due to the subject having been used in a previous lead experiment.

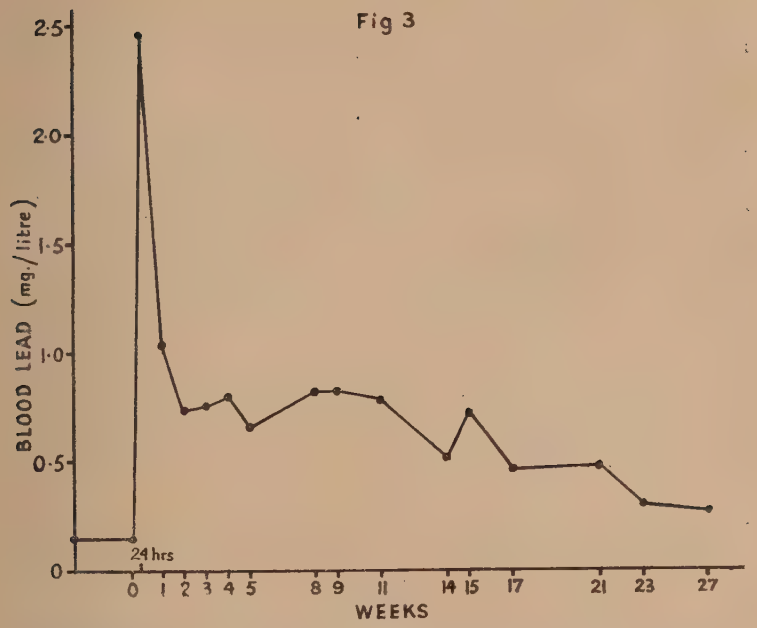

FIG. 3.—Blood lead values for calf No. 869, aged three months, given a single sub-lethal dose of lead (15·7 g.) as wet paint, *i.e.*, 0·12 g. Pb per kg. body weight. Survived. This illustrates the length of time taken for the blood lead level to return to normal.

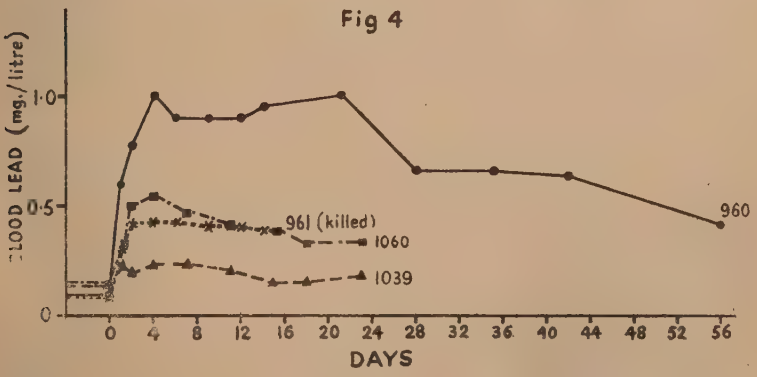

FIG. 4.—Blood lead values for four calves after a single dose of lead as the ore galena or as the metal. ●———● calf No. 960 aged seven and a half weeks, given 46·6 g. Pb as finely ground galena, *i.e.*, 0·5 g. Pb per kg. body weight. X– – – –X calf No. 961, aged seven and a half weeks, given 15·9 g. Pb as finely ground galena, *i.e.*, 0·25 g. Pb per kg. body weight. Killed on 16th day for analysis of tissues. ▲— — —▲ calf No. 1039, aged four months, given 100 g. Pb as shot, *i.e.*, 0·5 g. Pb per kg. body weight. ■— – – —■ calf No. 1060, aged four months, given 70 g. Pb as fine metallic dust, *i.e.*, 0·5 g. Pb per kg. body weight.

months after a single dose of 15·7 g. of lead in the form of wet lead paint. This very slow fall in the lead content of the blood is in agreement with the results obtained on sheep and rabbits, and has been discussed in the preceding paper.

From the blood data and from the data on the survival of the calves which are also given in Table III, it is possible to make comparative estimates of the amounts of lead absorbed from the various compounds administered. It would appear that lead ingested in the form of the phosphate, the oxide, the basic carbonate, and wet or dry paint, is equally toxic and results in comparable elevated blood levels when similar quantities are given. It can be concluded, therefore, that they are absorbed from the gut in similar amounts. In the case of lead sulphide, given as the finely ground ore galena, levels of lead in the blood stream did not rise to equivalent heights when comparable quantities were given, and the calves survived much larger doses. Similar results were obtained when large doses of lead as the metal were given. Some absorption, however, does occur, but it is much smaller than from the oxide, the basic carbonate, or the phosphate. This is shown by the blood and tissue levels in Fig. 4 and Table V respectively. In the case of the acetate, the results are conflicting as far as toxicity is concerned, and from the limited data presented here it appears that lead in this soluble form is no more toxic than the carbonate, oxide or phosphate. Indeed, literal interpretation of the actual data would make these three compounds more toxic than the acetate, although it seems difficult to credit this, and long-term chronic toxicity trials where lead as the acetate, carbonate and sulphide were used, show that this is not so. The blood lead levels indicate as rapid a rise in the lead content of the blood in the initial few hours after ingestion of the acetate but a more rapid fall than in the case of the other compounds used. The data show quite clearly, however, that there is no greater absorption of lead from this highly soluble lead compound than from the slightly soluble ones.

It is of interest that in the case of calf No. 856 (Table V) the very high blood lead value of 4·06 mg. per l. was observed 50 days after ingestion of a total of 236 g. lead as the basic carbonate in two periods spaced six weeks apart. In spite of this remarkably high value and exhibition of symptoms of acute lead poisoning this calf survived and was killed three and a half months later when the blood lead value had fallen to 1·36 mg. per l.

Tissues. Table IV shows the elevated lead content of selected tissues of calves which died after being given large quantities of lead. The results show that by far the highest concentrations were found in kidney cortex and liver tissue as would be expected from observations reported in the literature (Cantarow and Trumper, 1944). The cortex of the kidney always contained the highest concentration, a fact in agreement with results obtained by Behrens and Baumann (1933) and in acute experiments with rabbits (Blaxter, 1950*b*), while

the spleen, lung, heart and brain contained detectable but much smaller amounts. Results obtained by the slaughter of three animals given amounts of lead which were not sufficient to cause death are shown in Table V. It is difficult to compare these results directly with those given in the preceding table, since the blood data in Table III showed that absorption of lead after ingestion of galena and lead as the metal was smaller than after the other lead compounds. Calf No. 856 was not slaughtered until it was certain that the animal had survived the amount of lead administered, so the tissue concentrations refer to a much later date after dosage than do those for the calves in Table IV. Nevertheless, the data show (Table V) that a six-month-old calf can survive even when kidney cortex

TABLE IV

LEAD CONTENT OF SELECTED TISSUES OF CALVES WHICH DIED AFTER LARGE DOSES OF VARIOUS LEAD COMPOUNDS

Calf No.	*Age*	*Form of lead*	*Dose g./kg. body weight*	*Lead content in mg. per kg.*						
				Liver	*Kidney cortex*	*Kidney medulla*	*Spleen*	*Lung*	*Heart*	*Brain*
775	6 months	Wet white lead paint	0·84	75·4	253·0	32·0	5·7			1·3
781	6 months	Wet white lead paint	1·10	126·0	144·0	24·5	4·5			1·0
862	14 weeks	Wet white lead paint	0·25	45·0	297·0		2·2	3·6	1·0	<1·0
881	4 weeks	Dry paint flakes	0·23	15·5	200·0	4·6	1·0	2·3	<1·0	<1·0
865	10 weeks	Basic lead carbonate	0·24	34·0	230·0		3·3	3·3	1·3	1·2
891	7 weeks	Basic lead carbonate	0·22	27·0	260·0	4·0	2·3	2·4	1·3	1·4
922	7½ weeks	Lead oxide	0·25	24·3	132·0	4·2	<1·0	2·0	<1·0	<1·0
951	10 weeks	Lead phosphate	0·50	9·3	87·0	4·7	1·2			
976	6 weeks	Lead phosphate	0·25	11·1	50·5	3·0	1·7			
941	2 days	Lead acetate	0·35	9·7	72·0	5·5	3·0	2·3	1·0	1·5

lead is as high as 39 mg. per kg., 110 days after a large dose of lead, while from Table IV it is apparent that death can occur when the kidney cortex value is only slightly greater (50·5 mg. per kg.). If calf No. 856 had been killed a few days after dosing it is probable that the kidney cortex value would have been very much higher than 39 mg. per kg.

Faeces. Useful information can be obtained from a study of blood and faecal levels and illustrative data are given in Table VI showing values obtained after administration of sub-lethal doses of lead as galena and acetate. Faecal lead values obtained from 14 normal calves ranged from 1·5 to 30 mg. per kg. on a dry matter

TABLE V

LEAD CONTENT OF SELECTED TISSUES OF CALVES WHICH SURVIVED ATTEMPTS TO POISON THEM WITH LEAD COMPOUNDS

Calf No.	*Age*	*Form of lead*	*Dose g./kg. body weight*	*Slaughtered at days after last dose*	*Blood mg./l.*	*Lead content in mg. per kg.*						
						Liver	*Kidney cortex*	*Kidney medulla*	*Spleen*	*Lung*	*Heart*	*Brain*
856	6 months	Basic lead carbonate	*	110	1·36	6·5	39·0	3·7	2·0	1·0	1·0	2·9
961	7½ weeks	Galena (finely ground) ...	0·25	15	0·38	1·5	1·5	1·0	1·0	—	—	—
1060	4 months	Lead as the metal (fine dust) ...	0·5	14	0·32	3·3	3·7	—	—	—	—	—

* A total of 236 g. of lead had been ingested by this calf in two periods spaced six weeks apart.

TABLE VI

LEAD CONCENTRATIONS OF BLOOD AND FAECES AFTER ADMINISTRATION OF SUB-LETHAL DOSES OF LEAD AS FINELY GROUND GALENA AND AS THE WATER-SOLUBLE ACETATE

Calf 960	*Lead as galena given at the rate of 0·5 g. per kg. body weight. Total Pb 46·6 g.*		*Calf 936*	*Lead as the acetate given at the rate of 0·2 g. per kg. body weight. Total Pb 6 g.*	
Time after ingestion	*Blood Pb mg. per l.*	*Faecal Pb mg. per kg. dry matter*	*Time after ingestion*	*Blood Pb mg. per l.*	*Faecal Pb mg. per kg. dry matter*
Before	0·10	3	Before	0·08	15
2 weeks	0·96	1,220	1 day	1·22	103,800
3 „	1·0	1,168	2 days	1·04	14,800
4 „	0·66	146	3 „	0·90	1,100
5 „	0·66	45	4 „	1·06	568
6 „	0·64	20	9 „	0·78	64
8 „	0·42	11	14 „	0·70	30

basis with a mean of 12 mg. per kg. ; the range for 12 normal cows was 6 to 35 mg. per kg. with a mean of 13 mg. per kg.

The data in Table VI indicate that even when faecal lead values have returned to normal, blood values still remain abnormally high. This can be of considerable diagnostic value since in cases of suspected lead poisoning in the living animal, if both blood and faecal values are elevated then it can be assumed that ingestion of lead occurred within the recent past, that is, within three to four weeks ; or within two weeks if the soluble lead acetate has been ingested, although it is very unlikely that lead as the acetate would be encountered under farm conditions. If blood lead is high but faecal lead is normal, then ingestion probably occurred a considerable time before and there would be very little danger of the animal dying from lead poisoning. It would be possible, of course, to have a high blood lead value and a normal faecal value a few hours after ingestion of the lead compound and before unabsorbed lead had reached the anus, but in this case it would be too early to observe any suspicious symptoms in the animals and lead poisoning would therefore not be suspected. It is pointed out in the last paper of this series that in the calf there are often no apparent symptoms until an hour or two before death.

Experiments Involving the Continuous Administration of Lead

The object of this series of experiments was, as far as possible, to simulate the conditions which might occur on farms where cattle would be continuously ingesting small quantities of lead. Such conditions might arise by contamination of water supplies passing through lead pipes, the flaking of small quantities of lead paints from food receptacles and stalls, the ingestion of rations slightly

contaminated with lead from plumbiferous soil or from processing machinery, or the lodging of lead metal from lead shot, lead tags on feed sacks and other sources, in the reticulum of the animal.

The effect of lead ingested in the drinking water on blood and haemoglobin values is shown in Fig. 5. This calf, No. 778, aged

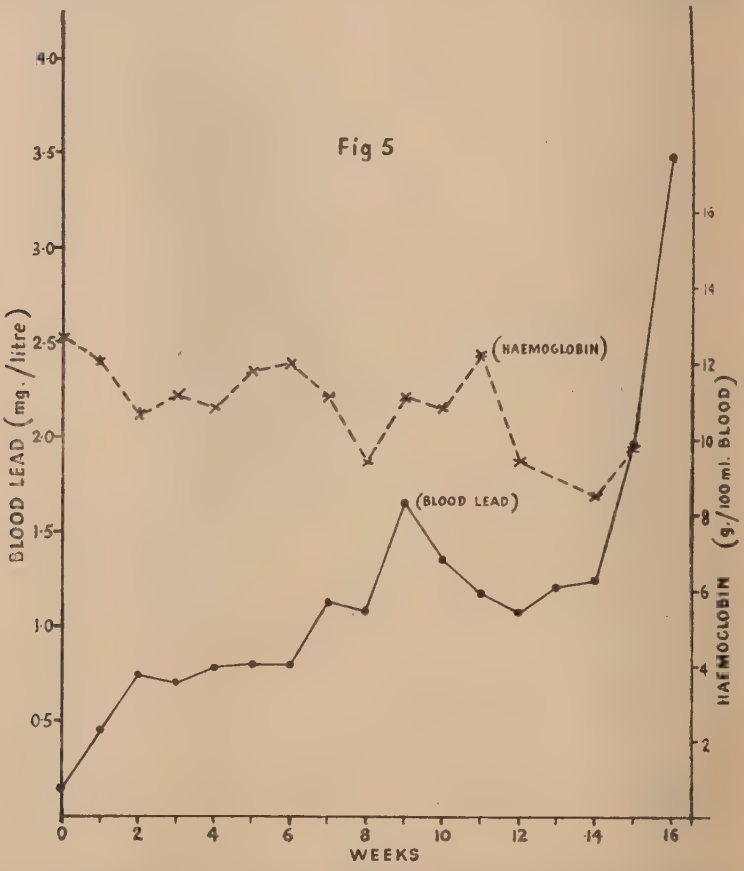

FIG. 5.—Haemoglobin and blood lead values for calf No. 778 (aged eight to ten months at commencement of experiment) given lead as the nitrate in solution in the drinking water at the rate of 100 p.p.m. Average daily intake was 2·3 g. Pb and approximate total intake over the experimental period of four months was 284 g. X – – – – X haemoglobin. ●———● blood lead.

eight to ten months at the commencement of the experiment, was given water containing 100 mg. of lead as the nitrate per l. It is extremely unlikely that farm water supplies would ever reach such levels except through gross contamination by industrial effluent. It is certain that such levels would never occur in water supplies flowing

through lead pipes. The calf survived for four months, and as shown in the graph, the Pb content of the blood rose over the course of two to three months to ten times the pre-experimental level. Immediately prior to death the blood Pb had risen to the high level of 3·5 mg. per l. The total lead ingested during the four-month experimental period was 284 g. of lead, equivalent to 2·3 g. per day. On the basis of its dietary intake of dry matter, this is equivalent to 500 mg. per kg. One sample of faeces taken after one month's treatment contained 800 mg. per kg., as compared with a pre-experimental value of 9·2 mg. per kg. In view of the limited absorption of lead from the digestive tract of the smaller ruminant, the sheep, (Blaxter, 1950*a*), and assuming that the digestibility of the ration dry matter was 60 per cent., this faecal value of 800 mg. per kg. suggests that there was an average intake of 480 mg. per kg. dry matter ingested at this time. This result implies that the small absorption of lead by the sheep also applies to cattle, and that faecal levels of lead can be used to give a fairly accurate estimation of lead ingestion.

At death, the lead content of the tissues of this calf was determined with the results shown in Table VII. It will be noted that

TABLE VII

THE EFFECT OF THE INGESTION OF DRINKING WATER CONTAINING 100 MG. Pb PER l. ON THE DISTRIBUTION OF LEAD IN THE SOFT TISSUE AT DEATH. CALF NO. 778

Tissue	*Pb content in mg. per kg.*
Liver	9·7
Kidney cortex	72·0
Kidney medulla	5·5
Spleen	3·0
Lung	2·3
Heart	<1·0
Skeletal muscle	1·0
Brain	1·5

the pattern of lead distribution is closely similar to that of calves dying rapidly after a single dose of a lead compound.

It was clear from the above experiment that the ingestion of 2·3 g. of lead daily as the nitrate resulted in death in a relatively short time.* Such a level of lead ingestion can hardly be regarded as a chronic poisoning, for in man the ingestion of only a few mg. daily

* These results were assembled for publication over two years ago. In view of the survival of two animals (Nos. 959 and 960) on a daily dosage of lead at the rate of 1 g. for the first year, 2 g. for the next six months, 3 g. for the next year and 4 g. for the last year, it seems possible that the high concentration of nitrate as well as the lead in the drinking water caused this animal to die in such a relatively short period.

eventually leads to clinically recognisable symptoms of plumbism. Two subsidiary experiments were carried out in which road dust from a plumbiferous area and tailings from an old lead mine were given to two calves. The results are shown in Table VIII. It is evident that the ingestion of these smaller amounts of lead over approximately the same period of time as the lead was given in the drinking water to calf No. 778 resulted in slight deposition of lead in the tissues. The tailings and dust contained only 0·2 and 2 per cent. of Pb respectively and the ingestion of 50 g. of this dust daily is probably at the limit of normal ingestion of soil- or dust-contaminated herbage for animals of this age. The daily amount given in each case was 10 g. for the first 14 days, 30 g. for the next 54 days and 60 g. for the remainder of the period (101 days in the case of the tailings and 57 days in the case of the dust).

TABLE VIII

THE EFFECT OF THE INGESTION OF SMALL QUANTITIES OF LEAD AS PLUMBIFEROUS SOIL DUST AND LEAD TAILINGS ON THE CONCENTRATION OF LEAD IN THE TISSUES

Calf No.	753	754
Length of treatment	169 days	125 days
Nature of material fed	Tailings	Dust.
Total amount of material given	11·4 kg.	5·3 kg.
Percentage Pb content of material ingested	0·2	2·0
Total lead ingested	17·1 g.	104·8 g.
Lead content in mg. per kg. of :		
Liver	2·9	6·9
Kidney cortex	3·2	10·7
Kidney medulla	1·0	1·1
Heart	<1·0	<1·0
Spleen	<1·0	1·4
Lungs	<1·0	1·0
Muscle	<1·0	<1·0
Brain	LOST	<1·0
Humerus head	51·5	
Humerus shaft	29·8	

To follow the effects of lead ingested in small quantities over much longer periods of time, the three experiments which are shown graphically in Figs. 6, 7 and 8 were commenced. The plan of these experiments was to give each animal 1 g. of lead daily for 12 months, one animal (No. 855) to receive the acetate, one (No. 959) the basic carbonate and one (No. 960) the precipitated sulphide. After 12 months dosage was increased at intervals of either six months or 12 months, these increases in dosage being shown on the graphs. The experiment with lead acetate has now lasted for almost three years, the other two for two. The animals were three to four months old at the commencement of the experiment and blood samples were taken at fortnightly intervals throughout. From the graphs it can be seen that these small amounts of lead all caused an

initial rise in the blood concentration, followed, as the calves grew, by a slow fall to a level " plateau " ten to 12 months after commencement of the experiment. Each increase in dose resulted in a slight rise in blood lead followed by a fall to a " plateau " again later except in the case of No. 960 after the first increase in dose at 12 months. This phenomenon of a large initial rise followed by a fall in blood lead concentration is most likely related to the fact that as dosage was at a constant rate, the dosage per kg. of body weight declined rapidly as the animal increased in weight. It is, however, important to note that the peaks were not established until the animals had been ingesting lead for two to three months, and that the initial plateau of blood lead after ten to 12 months was between 0·6 and 1·0 mg. per l. in all cases, that is, some five times the normal value. The haemoglobin content of the blood of these animals is of some interest especially in the case of No. 855 which is shown in Fig. 6. It can be seen that the haemoglobin value fell during the initial rise in blood lead and then adjusted itself to normal levels until the lead intake was increased to 2 g. daily. After that there was a steady fall over the next 15 months to the low level of

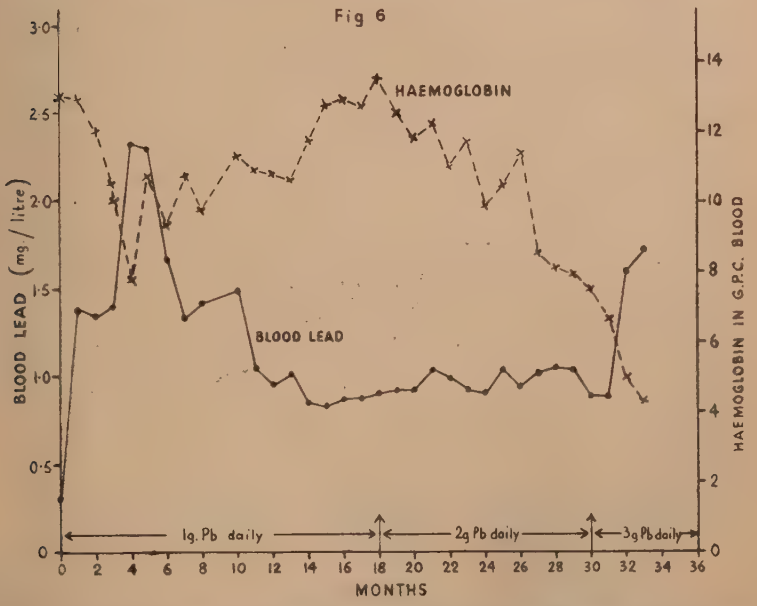

FIG. 6.—Calf No. 855. The effect on blood lead and haemoglobin values of the ingestion of small daily doses of lead as the water soluble acetate over a long period. X– – – –X haemoglobin. ●———● blood lead. Daily intake was 1 g. Pb for 18 months, 2 g. for the next 12 months, and then increased to 3 g. This animal was still alive at the end of the 33rd month but showed inappetence, loss of condition and weakness.

4·3 g. per 100 ml. blood. As stated before, this animal had been receiving lead acetate for nearly three years and in the last two months had fallen off badly in condition and was showing signs of chronic toxicity coinciding with the marked increase in blood lead and decrease in haemoglobin values. The behaviour of the haemoglobin curves in the other two animals (Figs. 7 and 8) is not so well defined and shows somewhat erratic variation throughout. These experiments are being continued until the animals die of lead poisoning.

To investigate the possibility of lead poisoning of the chronic type following the lodging of metallic lead in the digestive tract, two

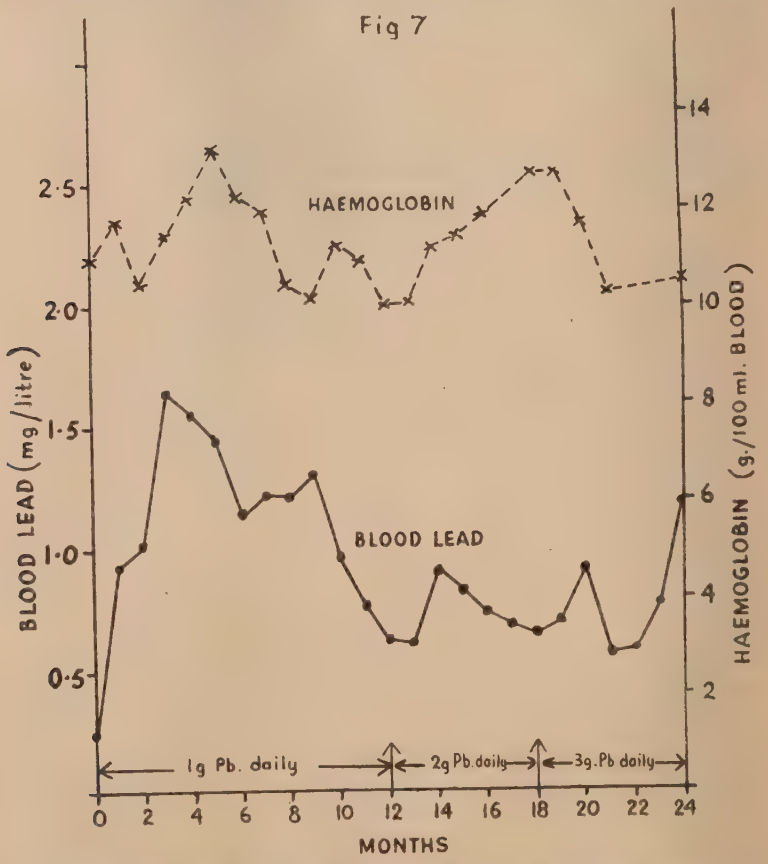

FIG. 7.—Calf No. 959. The effect on blood lead and haemoglobin values of the ingestion of small daily doses of lead as the carbonate over a long period. X – – – – X haemoglobin. ●———● blood lead. Daily intake was 1 g. Pb for 12 months, 2 g. for the next six months and then increased to 3 g. At the end of 24 months there were no toxic effects and the animal was in excellent condition. The experiment is being continued.

experiments were carried out and the results are shown in Table IX. Calf No. 1039 was given a total of 400 g. lead shot over a period of four months and it is of interest that 84 per cent. of this was recovered from the digestive tract when the animal was slaughtered five months after the last dose. Most of the shot were found in the reticulum and very few in the rumen.

The blood and tissue values in Table IX indicate that there was very little absorption from the metal and that the possibility of lead poisoning in the bovine from ingestion of metallic lead or the lodging of the metal in the tissues is extremely remote.

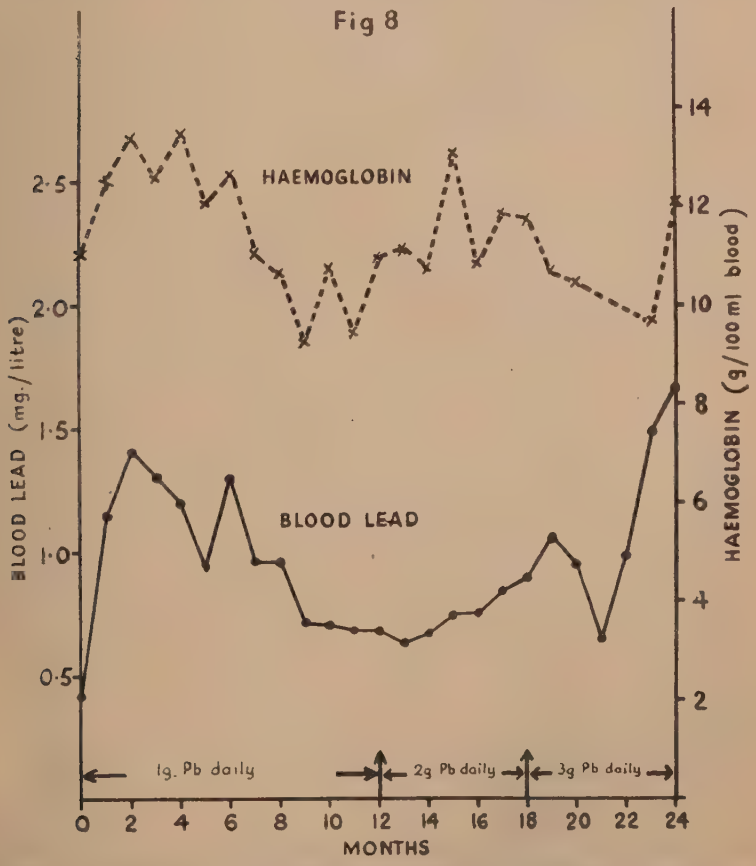

FIG. 8.—Calf No. 960. The effect on blood lead and haemoglobin values of the ingestion of small daily doses of lead as the precipitated sulphide over a long period. X– – – –X haemoglobin. ●————● blood lead. Daily intake was 1 g. Pb for 12 months, 2 g. for the next six months and then increased to 3 g. At the end of 24 months there were no toxic effects and the animal was in excellent condition. The experiment is being continued.

TABLE IX

THE EFFECT OF INGESTION OF METALLIC LEAD BY CALVES

Calf No.	1039	1060
Age at commencement	4 months	4 months
Form of lead	Shot	Dust
Monthly dosage per kg. body weight	0·5 g.	0·5 g.
Number of doses	4	4
Total lead given	400 g.	340 g.
Preliminary blood level Pb mg. per l.	0·13	0·13
Highest blood level Pb mg. per l.	0·23	0·51
Fate	Survived	Survived
Time between last dose and slaughter	5 months	2 weeks
Lead content of tissues mg. per kg. :		
Liver	1·7	3·4
Kidney cortex	2·9	3·8
Percentage of metal recovered from digestive tract ...	84	—

DISCUSSION

The discussion of these results in relation to the diagnosis and toxicology of chronic and acute poisoning is deferred until the final paper in this series (Allcroft and Blaxter, 1950).

The results obtained on blood and tissue concentrations of lead in calves are, however, of some interest from a comparative aspect. Blumberg and Scott (1935) dealing with cases of severe lead poisoning in human beings found that the lead content of the whole blood varied from 0·5 to 4·1 mg. per l., values which are in excellent agreement with those which are reported above. Some extremely high values—up to 10 mg. per l.—have, however, been recorded for human beings who have been continuously exposed to lead (Magnuson and Raulston, 1940 ; Smith *et al.*, 1938). Such values have not been found in calves even when very high doses have been given and even when blood has been taken at intervals which would cover the peak period of lead absorption from the digestive tract. It has already been pointed out that the normal levels of lead in the blood of calves, sheep and goats is considerably lower than the published data for man, even where these latter data have been obtained by modern accurate analytical methods. The data of Tompsett and Anderson (1935) which were obtained on normal people in this country indicate a range of from 0·4 to 0·7 mg. per l. This level of lead in the blood is but slightly lower than the plateau levels observed in calves ingesting 1 g. of lead every day in addition to the normal lead contamination of their diets. At this time, the body weight of the calves was from 500 to 700 lb., that is, four to five times the human weight. If comparable blood levels indicate comparable exposures to lead the minimum daily exposure of a human being calculated from the calf data would be in the region of 100 mg. per day. All available evidence based on analysis of water, food,

and contaminated air refute such an estimate, and put normal human intakes at less than one-fiftieth of this quantity. The most likely reason for this anomaly is that the human being absorbs a greater proportion of its dietary lead intake than does the calf. In this respect, it has been shown by Blaxter (1950,a) on the basis of metabolism experiments with sheep that the absorption of lead from small quantities in the diet is very small—1 to 2 per cent. These results are not in agreement with either those on mice or on rats, where absorption is considerably greater. In this respect, Monier Williams (1938), in reviewing the evidence on absorption of lead by man, has concluded that absorption is in fact rapid in the human being. It is also known that lead enters the systemic circulation and the tissues much more readily as a result of absorption from the respiratory tract than from the gastro-intestinal tract (Cantarow and Trumper, 1944) and inhalation of lead compounds is much more likely to occur in man than in farm animals. Information on lead absorption in human beings is scarce, but if the indirect data are interpreted as showing up to 50 per cent. absorption of small quantities, and if Blaxter's figure of 1 to 2 per cent. is correct generally for ruminant species, then to obtain similar blood levels in the calf as in the human being, 50 times as much lead would need to be administered per kg. of body weight. The fact remains, however, that the similarity of blood levels of lead when such widely differing amounts are ingested per kg. of body weight, indicates a greater relative tolerance to lead in the ruminant.

SUMMARY

Analytical data were obtained showing the normal concentration of lead in selected tissues and blood of cattle and sheep. It was pointed out that while the concentration of lead in the tissues was similar to that in the human being, the amount of lead in whole blood was considerably lower than in man.

When large quantities of lead were given to calves there was a marked and rapid rise in the blood lead level and if the animal survived the lead content of the blood did not revert to normal for many months.

Data on the concentration of lead in the blood stream has been used to compare the amounts of lead absorbed from various lead compounds by the calf. When given as the metal or the finely ground ore galena, lead was absorbed to a much smaller extent than when given as the acetate, the phosphate, the basic carbonate, the oxide and wet or dry paint.

Tissue concentrations of lead after ingestion of various lead compounds showed that the amount of lead in the kidney cortex was of specific diagnostic value.

Comparison of blood and faecal lead levels were made over a period of several weeks after ingestion of sub-lethal amounts of lead.

It is pointed out that such a comparison is of considerable diagnostic value in cases of suspected lead poisoning in the living animal.

Experiments on attempted chronic poisoning of bovines with lead are presented. Relatively large quantities, 1 to 2 g. daily, were ingested over a period of two years without ill effect.

REFERENCES

Allcroft, R., and Blaxter, K. L. (1950). *J. comp. Path.*, **60,** 209.
Behrens, B., and Baumann, A. (1933). *Z. exp. Med.*, **92,** 241.
Blaxter, K. L. (1950*a*). *J. comp. Path.*, **60,** 140; (1950*b*). *Ibid.*, 000.
Blaxter, K. L., and Allcroft, R. (1950). *Ibid.*, 133.
Blumberg, H., and Scott, T. F. McN. (1935). *Bull. Johns Hopkins Hosp.*, **56,** 32.
Cantarow, A., and Trumper, M. (1944). "*Lead Poisoning.*" The Williams and Wilkins Co., Baltimore.
Fenstermacher, R., Pomeroy, B. S., Roepke, M. H., and Boyd, W. L. (1946). *J. Amer. vet. med. Ass.*, **108,** 1.
Magnuson, H. J., and Raulston, B. O. (1940). *J. Amer. med. Assn.*, **114,** 1528.
Monier Williams, G. W. (1938). *Public Health Reports, Ministry of Health*, No. 88.
Shrewsbury, C. L., King, F. G., Barrick, E., Hoefer, J. A., and Doyle, L. P. (1945). *J. anim. Sci.*, **4,** 20.
Smith, F. L., Rathmell, T. K., and Marcil, G. E. (1938). *Am. J. clin. Path.*, **8,** 471.
Steyn, B. G. (1940). *Farming in S. Africa*, **15,** 350.
Tompsett, S. L., and Anderson, A. B. (1935). *Biochem. J.*, **29,** 1851.
White, E. G., and Cotchin, E. (1948). *Vet. J.*, **104,** 75.

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LEAD AS A NUTRITIONAL HAZARD TO FARM LIVESTOCK

V. The Toxicity of Lead to Cattle and Sheep and an Evaluation of the Lead Hazard under Farm Conditions

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INTRODUCTION

Previous papers in this series dealt with factors which determine the absorption of lead by cattle and by sheep and its retention in their bodies. Little attention was paid to the toxicological aspects of lead ingestion or to the practical importance of lead as a hazard under farm conditions. The present paper is concerned with these aspects, using experimental data obtained in this laboratory, together with the results obtained from the investigation of cases occurring under farm conditions, in which livestock losses were suspected to be due to lead poisoning and were dealt with as part of the routine diagnostic service. The data are presented in two parts, one relating to acute poisoning and the other relating to possible chronic poisoning.

ACUTE POISONING

Table I summarises data which have been obtained on the toxicity of lead in various forms when given in single doses to sheep and cattle under controlled conditions. The data indicate that

Table I

Toxicity of Lead when given in Large Single Doses to Cattle and Sheep in the form of Lead Acetate, Lead Carbonate, or Lead Oxide

Dose in g. per kg. of body weight	*Calves less than 60 days of age*	*Calves 2 to 4 months of age*	*Calves aged more than 4 months*	*Sheep aged one year or more*
Less than 0·2 ...	2 (survived)	1 (survived)	—	2 (survived)
0·2 to 0·4	5 (died) 2 (survived)	2 (died)	—	1 (survived)
0·4 to 0·6	1 (survived)	1 (died)	3 (survived)	1 (survived)
0·6 to 0·8	—	—	1 (died)	1 (died)
More than 0·8 ...	—	—	1 (died)	1 (died)

approximately 0·2 to 0·4 g. per kg. body weight of lead ingested on any one day, either as the acetate, the basic carbonate, or the oxide, is sufficient to cause death in calves up to four months of age, while in the case of older cattle and mature sheep, relatively larger doses are required. Of the survivors listed in the table, all except two showed symptoms of poisoning very similar to those observed in the early stages of poisoning in animals which subsequently died.

In poisoning of sheep symptoms occurred in the following pattern. A slight transient rise in body temperature—up to 1·5° F. —was noted the day after ingestion of the lead compound, followed later by progressive anorexia and reduced water consumption, persisting until death or disappearing with slow recovery. During this period of inappetence, loss of condition was very marked, the sheep became dull and lethargic, and weak as judged by ataxia of the hind quarters on prolonged standing. No tetany was observed, but there was often evidence of abdominal pain. Two of the sheep prior to death (including one poisoned by intravenous injection of lead acetate) showed elevated non-protein nitrogen concentration in the blood, which is not surprising in view of the kidney damage which occurs. From the results obtained in metabolism trials it was clear that in poisoned sheep, faeces output was reduced at least 24 hours prior to the failure of appetite, this constipation often being followed in severe cases by a dark, foul-smelling diarrhoea with a dry matter content of less than 20 per cent., as compared with the normal of 30 to 40 per cent. Urine volume varied markedly. With small amounts of lead polyuria occurred, while with large amounts oliguria persisted for as long as 36 hours.

In young calves acute symptoms in many cases markedly differed from those obtained with sheep. In general, however, dullness and inappetence occurred, as well as abdominal pain with constipation sometimes followed by diarrhoea. Usually these symptoms did not arise until two to three days after a fatal dose and, in some cases, the first symptom to be observed was sudden bellowing and staggering, with rolling eyes and frothing mouth. In this phase, which can last up to two hours prior to sudden collapse, the calf is greatly excited, running at the walls of the stall and attempting to climb them. Muscular spasms and tetany occur with increasing frequency until death. This series of symptoms represents the common clinical picture in accidental poisoning of the calf, and was not observed in sheep. The phase of extreme excitation may, however, be short and subsequent collapse rapid, so that the animal is merely "found dead."

At post-mortem examination all the sheep showed extensive inflammation of the gut and damage to the kidneys. Similar changes were not constant in calves; in particular, inflammation of the gut was not always observed and varied greatly in intensity. Kidney damage in many cases was only slight.

Field Cases of Acute Lead Poisoning

During a period prior to June, 1948, 190 deaths in cattle suspected to be due to lead poisoning were investigated. Of these, 91 were confirmed as positive, using tissue concentrations of lead as the diagnostic method. Many of the rest showed slightly abnormal, or even substantially abnormal amounts of lead in the tissues, but not sufficient to warrant an unequivocal diagnosis. The importance of lead poisoning as a cause of death in farm stock is indicated by the fact that 83 of these cases occurred in the six-month period ending June, 1948, and that 40 of these, involving 67 animals, were

TABLE II

INCRIMINATED SOURCE OF LEAD IN 80 CASES OF LEAD POISONING IN CATTLE IN THE FIELD

Source of lead	*Young calves*		*Heifers and cows*	
	Number of cases	*Number of animals*	*Number of cases*	*Number of animals*
Painted woodwork, including doors of pens, and walls on which plant had dried. Death due to chewing woodwork or licking off the flaking paint	33	53	0	0
Painted feeding troughs and utensils	2	5	0	0
Freshly painted walls and fences, tins of fresh paint left accessible ...	4	10	5	17
Contaminated rations of purchased food	1	*ca* 10*	1	*ca* 37*
Lead storage batteries	1	2	0	0
Painted canvas, roofing materials and tarpaulins	1	3	1	4
Painter's materials left on rubbish dumps, including putty and red lead but excluding tins of paint ...	1	2	2	5
Source not specified by practitioner	25	28	4	8
Totals	68	113	13	71

* This case involved the cows and young stock on one farm. A total of 47 animals died, with characteristic symptoms of lead poisoning, although post-mortem relicta were only sent for analysis from a few individuals.

positive. Since the number of veterinary practitioners who appealed for laboratory assistance was only 25, it is apparent that the actual loss of cattle in this country due to lead poisoning must be hundreds, perhaps thousands, every year.

In Table II the positive results from 80 cases are classified according to the suspected source of the lead which caused death. It is apparent that lead paint is the major source, for of those cases in which the origin could be definitely established, all except two were due to paint in one form or another—painted woodwork of stalls, freshly painted byres and buildings, paint tins left in accessible places, and

even in one case a discarded oil painting from the farmer's house, which had been licked down to the canvas. It is quite understandable that the poisoning cases in mature heifers and cows did not arise from licking and chewing painted surfaces. From consideration of the size of the cow alone, apart from the higher tolerance of older animals, it is obvious that very large areas of painted surface would have to be denuded to cause intoxication.

The one case of food poisoning given in the table was eventually traced to contaminated sugar beet pulp containing up to 1 per cent. of lead on the dry basis. The cause of the contamination could not be traced, owing to lack of co-operation on the part of the beet factory.

Three further cases of food contamination were encountered but are not included in the table. The first involved three farms, the death of three cows, and ill health of several more. The cause was reconditioned malting barley salvaged after a fire. It was heavily contaminated with burnt paint, lead oxides and metallic lead. Fortunately the source was identified in time to prevent further distribution of the feeding stuff. The second case involved the contamination of a cargo of copra cake by lead ore which was being transported by the ship at the same time. Contamination was suspected but since analysis only showed a maximum figure of 300 mg. per kg., and no ill effects were observed in experimental feeding trials at the laboratory, the cargo was released for incorporation in small quantities in compound cakes and meals. The third case was similar, involving a cargo of locust beans packed in lead contaminated sacks. Again, on the basis of analysis, this material was released for animal consumption. The lead content was only 25 mg. per kg. on the worst of several representative samples submitted.

CUMULATIVE POISONING

Table III summarises the experimental information obtained on the effect of long continued ingestion of lead by sheep and cattle. These results show that both non-pregnant sheep and cattle can survive up to 5 mg. of lead per kg. of body weight daily without showing any symptoms for one year at least and possibly for more than two. Increasing the dosage to 8 mg. per kg. daily was followed by death after 220 days in one ewe, but analyses of the preliminary results on the cattle it appears that 6 mg. per kg. daily may be tolerated without obvious ill effect for about three years. If the dry matter intake of ruminants be roughly estimated at 25 g. per kg. of body weight, the concentration of lead in the diet, as acetate or carbonate, which was tolerated for two to three years by these experimental animals, is of the order of 250 mg. per kg. This suggests that cumulative poisoning resulting from long-continued ingestion of subtoxic amounts of lead is rarely, if ever, likely to be encountered under natural conditions.

TABLE III

EFFECTS OF LONG CONTINUED ADMINISTRATION OF LEAD TO CATTLE AND SHEEP, IN RELATION TO POSSIBLE CUMULATIVE INTOXICATION

Class of animal	*Animal No.*	*Source of lead*	*Dose per day*	*Total ingested*	*Dose * mg./kg. body weight*	*Results*
Non-pregnant ewes	612	Acetate	100 mg.	30 g.	2	Remained normal for 12 months.
	616	Acetate	200 mg.	60 g.	4	Do.
	3	Acetate	300 mg.	90 g.	6	Do.
	4	Acetate	400 mg.	88 g.	8	Died after 220 days' continuous dosage.
Pregnant ewes	954	Acetate	50 mg.	2·5 g.	1	Died following an illness of ten days three weeks before lambing.
	960	Acetate	50 mg.	2·8 g.	1	Died after an abortion of a dead lamb one week before lambing.
	959	Acetate	50 g.	3·1 g.	1	Aborted dead lamb but survived.
	964	Acetate	50 mg.	3·3 g.	1	Aborted twin lambs but survived.
Six other ewes in this group, receiving lead acetate, lambed normally.						
Calves	959	Basic carbonate	1·0 g.	360 g.	5	Normal for 12 months.
			2·0 g.	720 g.	6	Normal for a further six months.
			3·0 g.	1,260 g.	6	Normal for a further six months.
	855	Acetate	1·0 g.	540 g.	5	Normal for 18 months.
			2·0 g.	1,260 g.	6	Normal for a further 12 months.
			3·0 g.	1,530 g.	5	Normal for a further three months before onset of anaemia.
	960	Precipitated sulphide	1·0 g.	360 g.	5	Normal for 12 months.
			2·0 g.	720 g.	6	Normal for a further six months.
			3·0 g.	1,260 g.	6	Normal for a further six months.

* The dosage per kg. of body weight in the case of the calves can only be an approximation since the animals were growing throughout the time during which the lead was being administered as a constant daily amount.

The results on pregnant ewes given in Table III, together with limited analytical data given in Table IV, suggest that the pregnant sheep is more susceptible and that abortion may follow continuous ingestion of lead in quantities quite safe for non-pregnant sheep. The dose given to these ewes corresponds to 50 mg. per kg. of dry matter ingested. The number of animals involved is small and they were not in good condition at the commencement of the experiment but a control group of ewes run simultaneously, receiving no lead,

showed no evidence of interference with gestation. A subsidiary experiment carried out the following year with five more robust ewes in which each was given 100 mg. of lead for the 15 days prior to lambing confirmed the placental transference of lead shown in Table IV. No abortions were observed in this short experiment,

TABLE IV

LEAD CONTENT OF THE TISSUES OF THE LAMBS AND FOETUSES OF EWES WHICH DIED DURING THE COURSE OF A PREGNANCY IN WHICH LEAD ACETATE WAS GIVEN, AND OF NON-PREGNANT EWES WHICH SURVIVED LONG CONTINUED INGESTION OF LEAD ACETATE

Ewe number and identification	*Daily ingestion of lead*	*Lead content in mg./kg.*			
		Liver (*wet tissue basis*)	*Kidney* (*wet tissue basis*)	*Bone* (*fat-free dry*)	*Blood mg./litre*
Ewe 612 slaughtered after survival for 12 months	100 mg.	4·6	4·7	76·9	0·34
Ewe 616 slaughtered after survival for 12 months	200 mg.	2·5	2·7	85·7	0·22
Ewe 960 died during pregnancy	50 mg.	17·1	—	—	—
Foetus from ewe 954 which died during pregnancy	50 mg.	37·7	—	—	—
Foetus aborted by ewe 959 ...	50 mg.	19·6	4·9	—	—
Twin lambs aborted by ewe 964	50 mg.	12·2	2·3	—	—
		3·5	2·8	—	—

but liver tissue of the new-born lambs showed lead values up to 10 mg. per kg. as compared with normal values of less than 1 mg. per kg.

The amounts of lead necessary to cause symptoms or death as a result of continuous ingestion may now be compared with the amounts of lead contained in the diets of animals grazing in normal and plumbiferous areas. Data on pasture composition are given in Table V. The lead content of uncontaminated pastures from areas where the soil does not contain much lead is uniformly low, and values exceeding a few mg. per kg. are always associated with contamination by soil dust. The samples cut with a motor mower on the Surrey farm were obviously contaminated. The herbage from Derbyshire was taken from farms in the vicinity of old lead mining areas and its lead content is uniformly much higher than normal samples from other areas. The contamination of the herbage from quarry dust and soil is very obvious. Such figures for lead in pastures, however, do not mirror the amount consumed by the animal, for the sheep is a selective grazer and dust contamination varies with the season. For this reason, samples of faeces were collected at intervals from the rectum of sheep grazing on two farms in the same area over a period of six months, covering the failing autumn grazing, the winter scarcity of keep, and the new growth

TABLE V

LEAD CONTENT OF PASTURES IN SOME AREAS OF GREAT BRITAIN

County in which farm was situated	*Number of fields sampled*	*Mean Pb content mg./kg. dry matter*	*Highest Pb content mg./kg. dry matter*
Surrey, Farm 1 (samples taken by hand)	3	3·9	5·0
Farm 1 (samples cut by motor mower)	3	14·6	15·7
Northumberland	2	5·3	7·5
N. Wales	1	5·7	
Surrey Aerodrome	4	5·9	7·2
Kent	4	25·9	36·6
Bedford	4	5·2	5·4
Cardiganshire	2	2·8	3·0
Derbyshire—			
Farm E. Jan., 1945 ...	1	94·0	
May, 1945 ...	6	29·8	34·7
Farm S. May, 1945 ...	7	34·9	47·4
Quarry dust settling on Farm S.	1	69·0	—
Cement works dust settling on Farm E	1	467·0	—

of herbage in the spring. It is clear from the data shown in Table VI that the lead intake per kg. of dry matter increases considerably as the herbage gets short in the late winter months. Assuming that the digestibility of the dry matter of the grazing was 50 per cent., then dividing the values listed in the table by 2 will give an approximation to the lead content of the herbage actually ingested, for, as previously demonstrated, the apparent absorption of lead is very small, and nearly all the lead ingested appears in the faeces (Blaxter, 1950). From the data, it is apparent that sheep in this area grazing

TABLE VI

LEAD CONTENT OF SHEEP FAECES ON TWO FARMS IN DERBYSHIRE WHERE THE LEAD CONTENT OF THE SOIL IS HIGH

Farm reference	*Date of sampling*	*No. of of samples*	*Mean lead content in mg./kg. of dry matter*	*Highest lead content in mg./kg. of dry matter*
Farm E ...	October, 1944	4	82·6	Samples pooled
	January, 1945	4	65·0	76·0
	March, 1945	5	109·7	140·6
	May, 1945 ...	7	28·3	37·8
Farm S. ...	October, 1944	4	111·0	Samples pooled
	January, 1945	4	131·7	152·8
	March, 1945	8	174·0	275·2
	May, 1945	8	43·8	58·6

near old lead mines ("raikes") can ingest a diet containing over 130 mg. per kg. in the late winter and down to about 20 mg. per kg. in the springtime.

Lead in soil, derived from weathered lead ore, is, however, less toxic than lead in the form of carbonate or acetate (Allcroft, 1950), and it is clear that even in these heavily contaminated areas, the effective lead content of the dietary never approaches levels which are sufficient to cause death after several years' ingestion. The ewes pass their lives on the pasture and lamb normally. It is, therefore, pertinent to ask whether the continued ingestion of these small quantities over many years is in fact reflected in any form of plumbism at all. Blood samples were taken from 40 ewes which ranged in age from one to six years and which had always lived on the same farms from which the faecal samples had been taken. The mean lead content of their blood was $0{\cdot}200 \pm 0{\cdot}010$ mg. per l. Allcroft (1950) has shown that the normal Pb content of sheep blood from other areas is $0{\cdot}139 \pm 0{\cdot}010$ mg. per l. The difference between these two values was shown to be statistically significant, and it can be concluded that there is a small real increase in the blood level of lead in this area. There was, however, no significant correlation between the age of the sheep and the amount of lead in the blood. As it has been shown that continued ingestion of lead in more available forms causes a marked rise in blood levels, it can be concluded that the small elevation in the Derbyshire area is physiologically insignificant.

DISCUSSION

The lead hazard to farm livestock is a very real one, but differs from the hazard to which the human subject is exposed. In the latter case, emphasis is usually placed on the danger of chronic poisoning from amounts of lead which would have no effect whatever on sheep and cattle. In dealing with lead poisoning in farm ruminants, the major, and perhaps only danger which occurs, appears to be that of *acute poisoning* by paint and heavily contaminated rations. This marked species difference has already been discussed in previous papers of this series. It is possible that there may be some interference with the health of sheep and cattle after many years on rations containing appreciable quantities of lead, but such interference has not yet been demonstrated. The suggestive results obtained with pregnant ewes in low condition need repetition on a larger scale to establish the probability that relatively small quantities do in fact cause abortion. The present experiments, however, indicate that chronic lead poisoning of cattle and sheep is extremely unlikely to occur under practical conditions of management in the economic lifetime of these animals.

The actual incidence of mortality in calves and cattle, as a result of the ingestion of sufficiently large quantities of lead over short periods of time, however, is sufficient to warrant considerable con-

cern. In almost all cases lead paint or painter's materials have been incriminated. The habits of young calves, especially pail-fed calves, of sucking and chewing the doors and walls of their pens and their feeding troughs necessitates the restriction of paint containing lead to only those places where it is completely inaccessible. There are at present available a range of paints which do not contain lead and these should be used to preserve woodwork in the immediate vicinity of calves. The disposal of rubbish, painted canvas, paint scrapings, and other material which might contain lead, should be such that no animals can gain access to it.

The diagnosis of lead poisoning in calves and cattle is best made by taking simultaneously into account the symptoms which have been described, the presence of a source of lead, the lead content of the blood and faeces if the animal survives and, if death occurs, the amount of lead in the soft tissues, especially the cortex of the kidney. Values higher than 50 mg. per kg. in the kidney cortex and 10 mg. per kg. in the liver are of definite diagnostic significance where there is collateral evidence of lead poisoning. It is sometimes difficult, however, to attempt to diagnose lead poisoning on the basis of analytical data alone. Thus, a value of 10 mg. per kg. for liver may only mean that the animal has ingested abnormal amounts of lead in the fairly recent past, not that death was in actual fact due to lead poisoning. Similarly, faecal levels of lead have to be interpreted with caution. A value of 200 mg. per kg. of dried faeces may only indicate the ingestion of plumbiferous soil dust, the lead of which is not easily absorbed (Allcroft, 1950), or it may represent the terminal stages in the elimination of a large amount of a more toxic lead compound such as the basic carbonate contained in paint. Where analytical evidence is the only evidence available, it has been our practice to regard kidney cortex values of 50 mg. Pb per kg. of wet tissue or more, and liver lead concentrations of 20 mg. per kg. or more, as sufficient to warrant a conclusion that lead poisoning has been the cause of death. Nevertheless, figures below this may be shown in cases of death following experimental administration of lead (White and Cotchin, 1948).

SUMMARY

Experiments in which the lethal dose of lead for calves and sheep was investigated using various compounds, showed that in the case of young calves 0·2 to 0·4 g. Pb. per kg. of body weight was sufficient to cause death in a few days. The fatal dose for older cattle and mature sheep was larger.

The symptoms of acute lead poisoning in cattle and sheep are described.

Field cases of acute poisoning are enumerated, and it is suggested that large numbers of calves die annually throughout the country as the result of the ingestion of toxic quantities of lead. Lead paint

and painters' materials were the most frequent cause of death, but cases also occurred through contamination of feeding stuffs.

It has been shown by experiments on continued daily ingestion of lead that the very substantial dose of 8 mg. per kg. body weight may be tolerated for many months, and that a level of 6 mg. per kg. of body weight per day may be tolerated for even three years.

On the basis of a single experiment it is suggested that much smaller amounts of lead, 1 mg. per kg. per day, are sufficient to cause abortion in the ewe if ingested during pregnancy by animals in poor condition.

Pasture levels of lead are discussed, and it is shown that the ingestion of herbage contaminated with plumbiferous soil dust in certain areas can result in the ingestion of more than 130 mg. of lead for every kg. of dry matter consumed. The average annual ingestion of lead by sheep in the plumbiferous area of Derbyshire is high, the amount being much greater in winter than in summer. Lead in this form, however, is relatively non-toxic and the lead content of the blood of sheep in this area suggests that there is no real danger of any form of plumbism.

It is concluded that cumulative lead poisoning rarely, if ever, occurs in ruminants, and that in this respect ruminants differ from human beings.

Diagnosis of lead poisoning of the acute type is briefly discussed, and it is emphasised that an unequivocal verdict on analytical data alone, without collateral evidence, is often difficult.

REFERENCES

Allcroft, R. (1950). *J. comp. Path.*, **60**, 190.
Blaxter, K. L. (1950). *Ibid*, 140.
Shrewsbury, C. L., King, F. G., Barrick, E., Hoefer, J. A., and Doyle, L. P. (1945). *J. anim. Sci.*, **4**, 20.
White, E. G., and Cotchin, E. (1948). *Vet. J.*, **104**, 75.

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NOTE ON THE USE OF DUBOS MEDIUM FOR CULTIVATION OF *MYCOBACT. TUBERCULOSIS* FROM MILK

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INTRODUCTION

Dubos (1945) introduced a liquid synthetic medium capable of supporting the growth of minute inocula of tubercle bacilli and maintaining this growth in diffuse form in the depth of the medium. The medium incorporates a synthetic non-ionic detergent called Tween 80 (a polyoxyethylene derivative of sorbitan mono-oleate) and Fraction V bovine albumen. The detergent is believed to enable the tubercle bacilli to establish contact with their nutrient medium and permit wetting of the so-called waxy surface. The albumen acts protectively by combining with growth-inhibitory substances such as oleic acid formed by hydrolysis of Tween 80.

Numerous publications (Dubos and Middlebrook, 1946 ; Dubos and Davies, 1946 ; Middlebrook, Dubos and Pierce, 1947 ; Dubos, 1947) have dealt with the growth of laboratory strains of human, bovine and avian strains of *Mycobact. tuberculosis* in Dubos medium, and Foley (1946) and Goldie (1947) have described its satisfactory use in isolations from human urine and sputum. The difficulty of finding tubercle bacilli in smears from tuberculous milk containing small numbers of organisms is well known and the use of Dubos medium suggests itself as a rapid aid to diagnosis and isolation. Although culture methods of diagnosis with human sputa have compared well with animal inoculation (Hoyt, Holtzwart, Kuntzner and Fisk, 1939 ; Whitehead, 1939 ; Sarans and Medlar, 1943), certain isolation of bovine strains, especially from milk, is often only possible by the slow method of guinea-pig inoculation (Holm and Lester, 1941).

We have examined a series of 46 milk samples diagnosed by microscopic examination of stained smears as being from cows with udder tuberculosis. Two orthodox methods of destroying contaminants, namely, 5 per cent. oxalic acid (Corper and Uyei, 1930) and 10 per cent. trisodium phosphate (Corper and Stoner, 1946), were tested with each milk sample and three different media, Dorset's egg, Loewenstein-Jensen and Dubos, used for subsequent cultivation. The liquid form of Dubos medium was used as we have some experience of this in laboratory work and solid modifications have not yet been evaluated. Smear preparation is also easier with a liquid

medium. A series of experiments were carried out, utilising artificially constituted tuberculous milk, to determine the reason for failure of Dubos medium in a small number of cases.

METHODS AND MATERIAL

Milk samples. These were despatched to the laboratory by veterinary inspectors of the Ministry of Agriculture. No special precautions were taken to prevent contamination and the samples were not collected under ideal conditions. The 46 samples had been diagnosed as tuberculous in varying degrees on the basis of examination of smears stained by the Ziehl-Neelsen method. Some contained very few bacilli. All samples were re-examined in the laboratory and 16 failed to reveal tubercle bacilli in stained smears, probably as a result of shaking in the post and time spent in transit.

Dubos medium. The solutions used in the preparation of the liquid medium are:—

Stock salt solution. Ten times final strength.

Potassium dihydrogen phosphate (anhydrous) 10·0 g.
Disodium hydrogen phosphate ($2H_2O$) 31·25 g.
Sodium citrate ($2H_2O$) 15·00 g.
Magnesium sulphate ($7H_2O$) 6·0 g.

Make up to 1 litre and store at 4° C. alone or over chloroform.

Tween 80. A batch* tested for use in Dubos medium. A 10 per cent. solution is prepared using warm water and a warm pipette.

Pronutrin. Enzymatic digest of casein† used as 10 per cent. solution to provide an amino-acid source of nitrogen.

Glucose-yeastrel solution. 2·0 g. glucose and 0·2 g yeastrel (commercial yeast extract) are dissolved in water and made up to 100 ml. Dispersed in ½ oz. McCartney bottles in 5 ml. amounts and sterilised at 15 lb. for ten minutes.

Albumen. 5 g. bovine serum albumen, Fraction V dissolved in 100 ml. water. Placed in waterbath at 56° C. for 30 minutes after neutralising to *p*H 6·9 to 7·0. This procedure destroys lipases in the albumen which hydrolyse Tween 80. Sterilised by passage through Seitz filter and used immediately.

To prepare 1 litre of stock medium add 5 ml. of 10 per cent. Tween 80 solution and 20 ml. of 10 per cent. pronutrin to 500 ml. distilled water. Add 100 ml. stock salt solution, dilute to 1 litre and mix. Distribute in 2·5 ml. quantities in small McCartney bottles and autoclave at 15 lb. for ten minutes. To each tube add 0·2 ml of albumen solution and 0·2 ml. of glucose-yeastrel. The medium can be stored in this final form for at least ten days at 4° C. Each batch of medium was tested with bovine, human and avian strains and rapid smooth dispersed growth obtained in three to four days.

* Obtained by courtesy of Messrs. Honeywell and Stein, 21, St. James's Square, London, S.W.1. The Tween series of detergents is made by The Atlas Powder Co., Delaware, U.S.A.

† Prepared by Herts. Pharmaceuticals, Welwyn Garden City.

The preparation of the medium is described in detail since Dubos himself has used several minor modifications. Pronutrin is used instead of Pepticase and Yeastrel instead of Vegex. This change was based purely on the availablity of materials.

Smear preparation. 10 ml. of milk were centrifuged at 3,000 r.p.m. for 30 minutes in McCartney bottles. The supernate was discarded, the base of the bottle thoroughly scraped with a 3 mm. platinum loop and the residue emulsified in the residual fluid. One or two loopfuls, depending on the amount of deposit, were smeared on microscope slides and stained by the Ziehl-Neelsen method.

Inocula. The emulsified sediment prepared as above was treated with 10 ml. of 5 per cent. oxalic acid for one hour at 37° C. (half an hour was insufficient). The oxalic acid solution was removed by two centrifugal washings with sterile saline and two loopfuls of the deposit were sown on Dorset's egg, Loewenstein-Jensen and in Dubos medium.

A further 10 ml. of milk was treated similarly, but using 10 per cent. trisodium phosphate in place of 5 per cent. oxalic acid, and the washed sediment was inoculated as above on the three test media. Treatment with trisodium phosphate for 24 hours is necessary to destroy contaminants, but no increased efficiency was obtained with 48 hours' treatment.

RESULTS

Tuberculous milk from field cases.

Destruction of Contaminants

Trisodium phosphate treatment of the 46 milk samples resulted in 13 contaminated cultures on Dorset's medium, eight on Loewenstein-Jensen and nine in Dubos medium. The corresponding contamination rates with oxalic acid treatment were eight, one and nil, respectively. Thus, treatment with oxalic acid is much superior to that with trisodium phosphate and the combination of oxalic acid treatment with Dubos medium or Loewenstein-Jensen superior to that with Dorset's egg medium.

Examination of Cultures

The presence of debris from the milk precluded the use of turbidity as a measure of growth in Dubos medium. Stained smears prepared from the whole uncentrifuged culture (2 drops using a 3 mm. loop) were therefore examined after seven, 14, 21 and 28 days' incubation. Smears from solid media up to 21 days yielded negative results and showed no visible growth. At 28 days, stained smears from all experimental media were examined, paying particular attention to suspicious patches on the solid media. A final microscopic and macroscopic examination of the solid media was made after three to four months' incubation.

The results are shown in the following table.

NUMBER OF CULTURES SHOWING ACID-FAST BACILLI IN SMEARS

Pre-treatment	10% *Trisodium phosphate*			5% *Oxalic acid*		
Medium	*Dorset's egg*	*L. Jensen*	*Dubos*	*Dorset's egg*	*L. Jensen*	*Dubos*
Incubation time						
7 days			2 (4%)			5 (11%)
14 „			5 (11%)			10 (22%)
21 „			13 (28%)			30 (66%)
28 „	10 (22%)	4 (9%)	14 (31%)	17 (37%)	16 (35%)	40 (88%)
3-4 months						
Macroscopic	12 (26%)	7 (15%)		23 (51%)	18 (40%)	
Microscopic	17 (37%)	11 (24%)		24 (53%)	25 (55%)	

Growth in Dubos medium occurred with all samples chiefly between 14 and 21 days and at 28 days the number of positive smears with the combination of 5 per cent. oxalic acid and Dubos medium exceeded those of the ten per cent. trisodium phosphate and Dubos medium. Solid media gave poor results using either method of pre-treatment. In no case did a solid medium give a positive result which was not also positive in Dubos medium.

Examination of solid media at three to four months merely served to demonstrate their inadequacy in detecting small inocula. Results were poor and inconsistent but oxalic acid pretreatment gave better results than trisodium phosphate, and Dorset's egg seemed rather better than Loewenstein-Jensen medium. An unexpected observation was that two oxalic acid-treated samples on Loewenstein-Jensen showed scanty organisms in smears where Dorset's and Dubos media had given negative results.

Sub-culture from Dubos medium gave in most cases readily dispersed growth in ten to 14 days, but as some culture bottles had dried off because of delay in attempting sub-cultivation, the results are not quoted in detail.

Attention was focussed on the six milk samples which had failed to show organisms in smears or culture. In each case the entire centrifuged deposit from 10 ml. of milk was inoculated into guinea-pigs and parallel samples of a further 10 ml. deposit were treated with oxalic acid, washed and 2·5 ml. of sterile Dubos medium added. Thus, except for the oxalic acid treatment, the inoculum into Dubos medium and into the guinea-pigs was identical, both receiving equal numbers of organisms. At post-mortem all six guinea-pigs showed lesions of tuberculosis, while the Dubos medium remained negative for acid-fast organisms at seven, 14, 21 and 28 days. Clearly Dubos medium fails to detect tubercle bacilli in milk where these are still demonstrable by guinea-pig inoculation.

Milk containing added tubercle bacilli.

The failure of Dubos medium to detect tubercle bacilli in 12 per cent. of samples examined may result from unsuitability of the medium itself, from pretreatment of the sample with oxalic acid solution or from the use of an inadequate inoculum. To investigate these factors an artificial tuberculous milk was prepared from a smooth suspension of bovine tubercle bacilli and milk obtained from a case of streptococcal mastitis. The opacity of the bacillary suspension was adjusted to Brown's tube 2, and 2 vols. of the suspension added to 8 vols. of milk. Serial ten-fold dilutions were prepared, using the same milk as diluent to ensure constant cellular content. Each dilution was treated as a separate sample to be examined by smear, biological and cultural methods, all treatment being done in duplicate.

10 ml. of each dilution were centrifuged and smears from the sediment stained by Ziehl-Neelsen. Bacilli could be detected in a dilution of 10^{-4} of the original milk. The entire deposits from further 10 ml. quantities were injected intramuscularly into guinea-pigs, with and without pretreatment for one hour with 10 per cent. oxalic acid solution. Treated deposits produced lesions in guinea-pigs at a dilution of 10^{-5}, and untreated at 10^{-8}. Thus oxalic acid exerts a significant lethal effect on tubercle bacilli when used to eliminate milk contaminants. Finally, two loopfuls of oxalic acid treated deposit from 10 ml. of each dilution were inoculated into Dubos medium and 2·5 ml. of the latter added to the residual deposit. No difference could be detected between these two methods, growth occurring in milk dilutions of 10^{-1} to 10^{-2}. Thus, the method used with tuberculous samples secured an adequate inoculum. The experiment repeated with a different bovine strain of tubercle bacillus gave substantially the same results.

With fresh artificial tuberculous milk, therefore, guinea-pig inoculation is superior to smear examination and the latter superior to culture methods, including that utilising oxalic acid and Dubos medium in which the oxalic acid treatment has a deleterious effect on tubercle bacilli.

DISCUSSION

The use of Dubos liquid medium, combined with oxalic acid for destroying contaminants, fails to detect minimal numbers of organisms still capable of infecting the guinea-pig, but gives much better results than solid media. Our results with milk containing added tubercle bacilli and the successful reports of Foley (1946) and Goldie (1947) suggest that while Dubos medium in itself may prove satisfactory, destruction of contaminants without simultaneous destruction of tubercle bacilli is still a problem. The most favourable conditions would arise by collection of the sample under as sterile conditions as possible, minimal chemical treatment of the

milk and, perhaps, incorporation of penicillin in the medium. The final evaluation of Dubos medium in combination with a sterilising agent such as oxalic acid will require careful attention to these factors. If the medium itself proves at fault improvement might be obtained by purification of the Tween 80 but such elaboration seems undesirable in a medium for routine use. No attempt has been made to examine the value of solid media incorporating Tween 80, or of slide culture enrichment methods. Milk itself might prove a natural substitute for Dubos medium and capable of being used for slide cultures of tubercle bacilli.

SUMMARY

Oxalic acid is more efficient than trisodium phosphate for the destruction of contaminating organisms in milk suspected of containing tubercle bacilli.

Dubos medium is more efficient than solid media for the isolation of tubercle bacilli from milk.

Dubos medium fails to detect tubercle bacilli in samples containing minimal numbers of organisms. Possible reasons for this failure are discussed.

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REFERENCES

Corper, H. J., and Stoner, R. E. (1946). *J. Lab. clin. Med.*, **31,** 1364.
Corper, H. J., and Uyei, N. (1930). *Ibid.*, **15,** 348.
Dubos, R. J. (1945). *Proc. Soc. exp. Biol. and Med.*, **58,** 361 ; (1947). *J. exp. Med.*, **85,** 9.
Dubos, R. J., and Davis, B. D. (1946). *Ibid.*, **83,** 409.
Dubos, R. J., and Middlebrook, G. (1946). *Amer. Rev. Tub.*, **51,** 334.
Foley, G. E. (1946). *Proc. Soc. exp. Biol. and Med.*, **62,** 298.
Goldie, H. (1947). *Ibid.*, **65,** 210.
Holm, J., and Lester, V. (1941). *Acta. Tuberc. Scandinav.*, **16,** 3.
Hoyt, A., Holtzwart, F., Kuntzner, B., and Fisk, R. (1939). *J. Lab. and clin. Med.*, **25,** 88.
Middlebrook, G., Dubos, R. J., and Pierce, J. (1947). *J. exp. Med.*, **86,** 175.
Sarans, K. T., and Medlar, E. M. (1943). *Amer. Rev. Tub.*, **48,** 297.
Whitehead, H. G. (1939). *Ibid.*, **39,** 540.

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FIELD TRIALS WITH *CORYNEBACTERIUM PYOGENES* ALUM-PRECIPITATED TOXOID

By

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INTRODUCTION

Corynebacterium pyogenes forms a filterable haemolytic toxin which is convertible to toxoid with formalin; its antigenic potency is increased by precipitation with alum and the intramuscular injection of this product induces the formation of antitoxin in the sera of injected animals. The antitoxin content of sera may be measured by *in vitro* tests utilising, for comparative purposes, standard antitoxin containing a specified number of "X" units per c.c. (Lovell, 1937, 1939*a*, 1941). Alum-precipitated toxoid has been used in the field with a view to ascertaining its value both as a prophylactic and as a therapeutic agent in bovine summer mastitis (Brown and Stuart, 1943; Weitz, 1949).

During the summers of 1942-45 a number of cattle in Ulster were injected with toxoid and a sufficient number of control animals in the same herds were observed. A brief record of the incidence of summer mastitis in the animals under observation and of the antitoxin content of the sera of a representative number is given.

METHODS EMPLOYED

Preparation of the alum-precipitated toxoid

A toxigenic strain of *C. pyogenes* was grown in flasks of broth containing meat for 48 hours at 37° C. Films were prepared and stained and cultures on blood agar made to ensure that no contamination existed. The meat-broth-medium cultures were filtered through muslin and Seitz filters, and the filtrate tested for its haemolytic activity and for sterility. Formalin was added to a concentration of 0·4 per cent. and the formalinised toxin returned to the incubator for four days. After testing for its conversion to toxoid by its inability to lyse red blood cells a sufficient amount of 10 per cent. potash alum was added to give a final concentration of 0·7 per cent. The flasks of toxoid were then placed in the ice chest and the supernatant fluid decanted and replaced by sterile saline daily for three days: the flasks were slightly agitated each time and

finally the supernatant was decanted so as to reduce the total bulk to about one third of its original volume. This was then preserved with merthiolate and tested for its combining power with antitoxin (Lovell, 1941) and for its antigenic potency in rabbits.

Inoculation and observation of animals in the trial

Herds of cattle were selected mainly on the basis of willing co-operation of the owner. Treated animals were given intramuscular injections of alum-precipitated toxoid and the size and number of doses varied in the different years. It was hoped to protect the animals during the "summer mastitis season" and the injections were therefore begun in April, May or June. During 1942-43 the injections were given at monthly intervals, but during the last two years a fortnightly system of injection was adopted. A number of vaccinated and control animals were bled at intervals and their sera examined for *C. pyogenes* antitoxin by the *in vitro* test. Observations were made on the incidence of summer mastitis occurring in the selected herds and in most cases this was confirmed by bacteriological examination of the udder secretion.

RESULTS

Incidence of infection

The incidence of summer mastitis in the animals under observation is shown in Table I which also gives details of the dosage given and the years in which the observations were made.

TABLE I

Year	Number and size of inoculations	Vaccinated			Controls		
		No. observed	*No. of cases*	*Morbidity percentage*	*No. observed*	*No. of cases*	*Morbidity percentage*
1942	2 × 10 c.c.	439	6	1·4	487	11	2·3
1943	10 c.c. + 2 × 5 c.c.	446	8	1·8	477	9	1·9
1944	2 × 5 c.c. + 10 c.c.	231	8	3·5	226	11	4·9
1945	3 × 4 c.c.	1,007	18	1·8	832	16	1·9
Totals		2,123	40	1·9	2,022	47	2·3

There is no evidence to suggest from the figures presented that the injections of toxoid influenced the incidence of summer mastitis in the observed herds. In 1942, seven of the eleven cases in the control group developed early in the season, namely between July 20th and August 4th ; the first case in the vaccinated group occurred on August 4th.

In 1944, several of the cases in the vaccinated group developed before the second or third inoculations had been given. It is therefore hoped that if and when further trials are made of a prophylactic against summer mastitis, strict attention will be paid to the number and spacing of doses. It is not easy to maintain a high level of immunity in cattle by means of a non-living prophylactic as judged by an *in vitro* serological test. The injected cattle did not maintain a prolonged high level of antitoxin in their blood serum and this is shown below.

Antitoxin content of sera of immunised and control cattle

During the first three years representative samples of cattle sera were examined for their antitoxin values. The samples were collected at intervals from immunised and from control animals and the test employed was an *in vitro* one whereby the antihaemolytic activity was compared with a standard serum containing a specified number of "X" units per c.c. The results are given in Figs. 1, 2 and 3: the mean-antitoxin values of the animals in each herd or group have been calculated and plotted accordingly.

The mean antitoxin values in the sera of vaccinated and control cattle in five herds are shown graphically in Fig. 1. After two or three months the titres fall in the vaccinated animals until they approximate to the control ones. There is no evidence of a pro-

FIG. 1

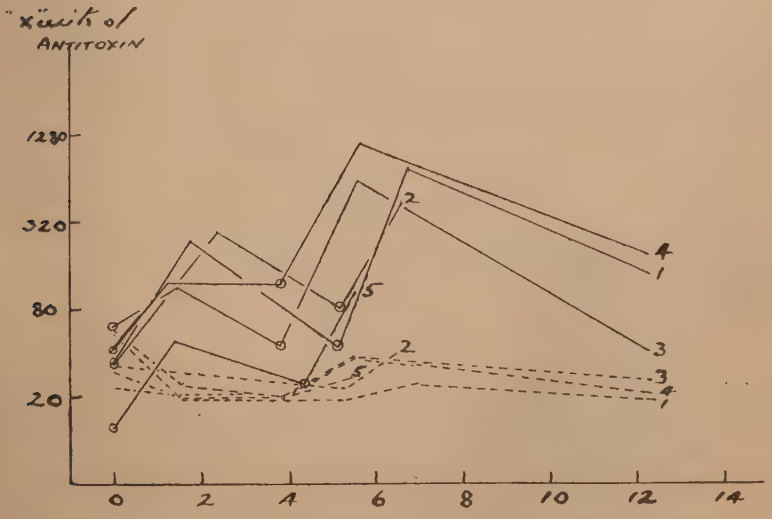
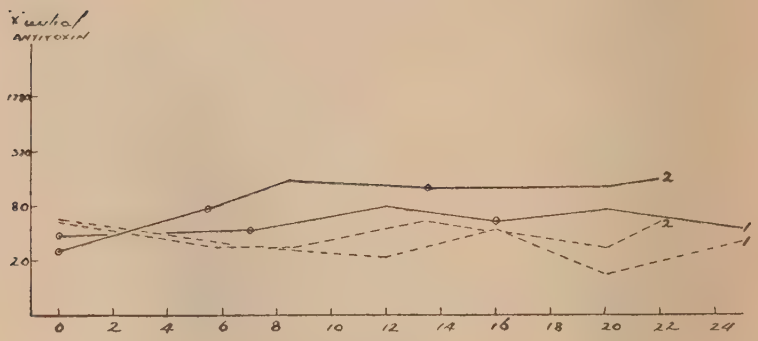
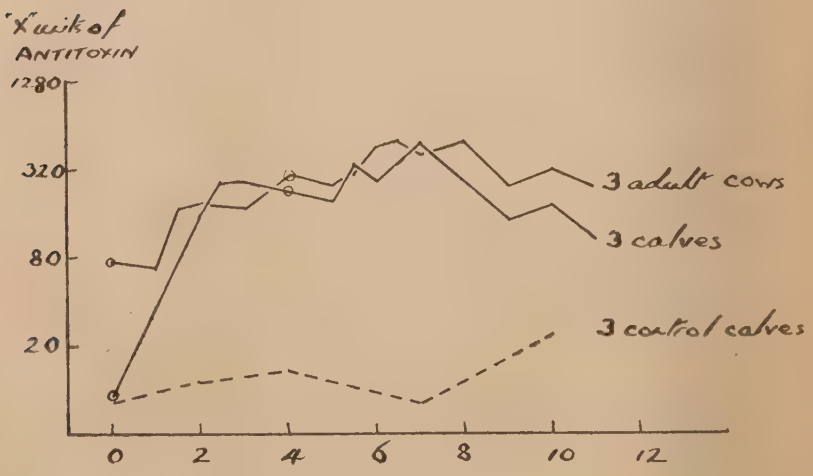


FIG. 2



longed high level of antitoxin content as was expected by using an alum-precipitated toxoid. With three doses of toxoid, as in Fig. 2, a high level was not obtained and maintained. All samples of toxoid used were tested for combining power and antigenic potency and one is forced therefore to consider the cow as being an unsatisfactory animal for immunisation purposes with toxoid. In Fig. 3 there is some evidence of a rapid rise in the sera of calves which have a low initial antitoxin content ; but the downward trend began after about two months.

FIG. 3



DISCUSSION

Owing to the widespread distribution of *C. pyogenes* in some animal species (Lovell, 1939*b*) the control of summer mastitis is unlikely to be effected by elimination of infection. Prophylactic immunisation or effective therapeutic agents are therefore necessary. It was with this in mind that the trials in Ulster were made and although the results obtained are not encouraging, a record of our experiences should help others in their observations. Weitz (1947) attempted to devise a system of dosage whereby the antitoxin content of cattle sera could be raised and maintained at a high level. Three doses at bi-monthly intervals gave the best results but in his field trials (Weitz, 1949) he was not able to produce evidence of any protective action of the toxoid. He suggests that the antitoxin induced may not be the antibody concerned in protection ; we would in part agree but feel that whilst the antitoxin induced may play no part in reducing the incidence of infection it may help in the reduction of mortality. This is borne out by the results of Brown and Stuart (1943), who found that toxoid when used therapeutically did not save infected udders but it reduced the death rate and influenced favourably the swollen and painful joints which commonly accompany summer mastitis.

SUMMARY

During the summer months of 1942-45, field trials were made in Ulster in order to assess the prophylactic value of *C. pyogenes* alum-precipitated toxoid against summer mastitis. There were 40 cases of infection in 2123 immunised cattle and 47 in a control series of 2022 : percentages of 1·9 and 2·3 respectively.

As judged by the time distribution of the cases and the antitoxin responses, there is need for a study to be made of the general problem of antibody response to non-living vaccines in cattle.

ACKNOWLEDGMENTS

We record our thanks to Dr. H. G. Lamont and Mr. P. L. Shanks for some of the earlier inoculations and to those veterinary surgeons who assisted in this field study ; without their help the trials would have been impossible.

REFERENCES

Brown, J. A., and Stuart, J. D. (1943). *Vet. Rec.*, **55,** 315.
Lovell, R. (1937). *J. Path. Bact.*, **45,** 339 ; (1939*a*). *Ibid.*, **49,** 329 ; (1939*b*). *Vet. Rec.*, **51,** 1081 ; (1941). *J. Path. Bact.*, **52,** 295.
Weitz, B. (1947). *J. comp. Path.*, **57,** 279 ; (1949). *Vet. Rec.*, **61,** 123.

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A DIRECT CULTURE METHOD FOR THE EXAMINATION OF MILK SAMPLES

By

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INTRODUCTION

Many direct and indirect methods have been used for the examination of milk samples for the detection of bacterial infection of the udder. Direct methods are preferred at this laboratory but have serious limitations. Samples from a distance are often unsuitable for examination and at best doubts arise about the exact significance of the types and numbers of organisms present.

METHODS

In an attempt to overcome these difficulties, experiments were made to devise a method whereby the bacterial content of the sample on arrival at the laboratory would be similar to that at the time at which it was taken. Of the earlier methods tried, the most promising involved the use of a tube containing a strip of sterile filter paper separated from calcium chloride by a thin layer of cotton wool. The paper, attached by two pins to the cork, was soaked in milk taken directly from the teat, shaken and replaced in the tube. The paper dried completely in a few hours, the rate of drying being proportional to the ambient temperature. At the laboratory, the strip was rubbed up in broth, a portion of which was plated while the remainder was incubated. Unfortunately, on account of the amount of work involved, the method was not satisfactory for large numbers of samples.

Reports that other workers were transferring milk directly from the cow to media in tubes or bottles led us to try similar methods. A wide-mouthed flat media bottle described by Neave, Taylor and Lawes (1949) was used. It was not entirely satisfactory partly because we wished to avoid any method which did not allow a direct view of the surface of the medium, and partly because this type of bottle was not available in quantity. A number of jars and bottles of different shapes and sizes were used and we finally decided on a jar of the face cream type, 4 cm. deep and about 6 cm. wide, with a screw aluminium lid. At first, blood agar in a thin layer was used but the convex bottom of the jar caused difficulties. In order to obtain a flat surface a layer of plain agar was poured in first and on top of this a thin layer of blood agar. The jars were incubated before use to test for sterility and to dry the agar so as to control spreading organisms and make it more absorbent. These jars were satisfactory except for the growth of certain contaminants

of the spreading type, which were to some extent inevitable with the exposure caused by the use of a jar with a wide mouth, and some discoloration of the blood agar by the milk. These jars which are virtually small media plates can be stored and are valuable for all culture media.

The original intention was to develop a method which would detect all organisms in the milk. Owing to the risk of the spreading type of contaminants and to the fact that the principal organisms likely to be encountered were streptococci there were obvious advantages in using a selective differential medium. Various media were employed but it was finally decided that a substrate containing thallium sulphate or thallium acetate in a strength of 1 : 3,000 was the best since it inhibited most contaminating organisms unless they were present in excessive numbers. In practice Edwards medium with the addition of this salt proved to be the most satisfactory for the detection of *Str. agalactiae* which was the main end in view. Other streptococci, however, were also recovered.

Routine sampling method adopted

The udders are not washed unless very muddy but if washing is necessary, they are dried before sampling. The ends of the teats are cleaned thoroughly using a 1 per cent. solution of Cetavlon (Cetyltrimethylammonium bromide) and then dried. The foremilk is not discarded but a small quantity is drawn from all four teats on to the surface of the medium. The jar is rocked to run the milk back and forward over the medium and the excess decanted into a bucket. The screw cap, containing the absorbent pad, is then put on and the jar placed in an inverted position so that any remaining milk can be absorbed by the pad. On arrival at the laboratory, the samples are incubated for 48 hours at 37° C., and the numbers and kinds of colonies recorded.

RESULTS

Comparison of the jar method with (a) direct plating and (b) plating after incubation

Herds were chosen which were known to be infected with *Str. agalactiae*. The method just described was compared with (a) direct plating of 0·1 ml. of whole milk on to 5 per cent. blood agar and (b) a preliminary incubation followed by plating of a loopful of gravity cream on to Edwards medium.

In the first few herds, the samples were taken alternately, *i.e.*, in the first cow the jar was used first, and in the second cow the McCartney bottle sample was taken first, and so on. This was discontinued after four herds, as no difference in the results could be observed, and subsequently the jars were always used first. The results for 13 herds are given in Table I. It will be seen that the jar method of sampling picked out more infected cows than did

direct plating but less than the method of preliminary incubation of the milk. Whilst the superiority of the jar plating method over direct plating for the detection of *Str. agalactiae* appears clear, the advantage of using incubated milk is far less significant. It has been our experience that those samples which are positive only after preliminary incubation frequently do not indicate infected cows but that the streptococci originate from the skin infection on the surface of the teats or are transferred from another infected cow through faulty technique in sampling.

TABLE I

DIRECT MILK SAMPLING ON TO SOLID MEDIUM (THALLIUM-SULPHATE JAR) COMPARED WITH PLATING FRESH AND INCUBATED MILK

		Str. agalactiae			*Str. dys-galactiae*			*Str. uberis*			*Haem. Staphs.*		
Herd	*No. of cows*	*T/S jars*	*Fresh milk*	*Inc. milk*	*T/S jars*	*Fresh milk*	*Inc. milk*	*T/S jars*	*Fresh milk*	*Inc. milk*	*T/S jars*	*Fresh milk*	*Inc. milk*
A	22	11	7	10	—	1	2	—	2	—		1	
B	39	8	7	11	3	3	3	—	—	—		—	
C	40	23	20	22	1	2	2	1	2	—		3	
	42	20	19	17	—	2	2	1	2	—		3	
D	33	7	2	9	—	1	—	1	1	—		2	
	35	2	1	10	—	—	—	2	1	2		3	
	36	11	4	4	—	—	—	2	—	2	Inhibited	—	Inhibited
E	27	3	2	6	—	—	—	1	—	—		1	
F	28	21	16	15	—	—	—	1	1	2		1	
G	23	8	1	12	—	—	—	2	1	2		—	
H	43	11	11	13	—	—	—	—	—	—		—	
I	40	12	6	12	1	1	3	3	2	3		2	
J	30	7	6	8	2	1	2	2	1	2		2	
K	41	17	17	20	—	—	—	—	—	—		—	
L	35	4	4	4	—	—	—	2	2	2		—	
M	43	22	17	23	—	—	—	2	1	4		4	
Totals	557	187	140	196	7	11	14	20	16	19		22	

The main advantage of this method is that work in the laboratory is reduced to a minimum. In practice, we have found that we have increased our weekly capacity for dealing with samples four or five times. A further advantage is that samples sent from a distance arrive at the laboratory without excessive contamination. In preliminary work, samples were taken and held at room-temperature up to six days or were sent a lengthy journey by rail before being placed in the incubator. Contamination was not increased and the number of infected samples detected was the same as in duplicated sets placed in the incubator within one hour of sampling.

This medium keeps satisfactorily for at least two weeks after preliminary incubation, but should be stored in a cool dry place. It must not be put in a cold store as the thallium salts crystallise out of the medium.

Identification of colonies on jars

Before this method could be adopted in large-scale work for the examination of milk samples for the presence of *Str. agalactiae*, a quick but reliable test for confirming the identity of this organism was essential. In a previous method used at the laboratory when each milk sample was examined firstly by plating on to 5 per cent. blood agar and secondly, after incubation, on to Edwards medium, one examination checked the other, but in the direct sampling method no such check existed. Additional check testing was therefore essential either by precipitation tests with group sera or by biochemical tests.

Various other methods were tried and discarded either because the work involved was too time-consuming or the results obtained were not sufficiently accurate. Maxted's (1948) method for the preparation of suitable extracts by lysing the Streptococci with an enzyme produced from the growth of *Streptomyces albus* much reduced the work of preparing extracts of streptococci but difficulties were encountered in the regular preparation of good enzyme solutions.

Christie, Atkins and Munch-Petersen (1944) described a simple but accurate method of identifying *Str. agalactiae*. They found that group B streptococci are capable of completing the lysis of the blood in the partially lysed zone produced when β toxin producing staphylococci are grown on a blood-plate. In a further communication (1945) they reported that 200 strains of group B streptococci of both human and bovine origin induced this phenomenon whilst 395 strains from other Lancefield groups and 20 ungrouped strains comprising *Str. uberis* and *Str. dysgalactiae* failed to do so.

We have tried out this method but in our hands the results have not been so clear cut. While we have not yet discovered a group B streptococcus which fails to produce the completion of the lysis in the β toxin zone, it has been our experience that various other organisms possess similar powers. These comprise some non-haemolytic staphylococci, some micrococci, a few strains of *Str. dysgalactiae* and 10 to 20 per cent. of strains of *Str. uberis* (identified by means of their biochemical reactions). All except *Str. uberis* can be recognised by their different cultural appearances. For the identification of *Str. uberis* it is now our practice to add aesculin to the blood-plate, because by this means we can differentiate it from *Str. agalactiae*.

Every streptococcus recovered after incubation of the thallium sulphate jars is now further identified by this method. A β toxin producing staphylococcus is streaked down the middle of a blood-plate containing 0·1 per cent. aesculin. The various streptococci to be identified are inoculated at right angles to this streak on either side and close enough for growth to occur within the β toxin zone of haemolysis. Ten strains for test are inoculated on each plate and the results are read after overnight incubation at 37° C.

Organisms other than Streptococci

Because this medium is essentially a selective one for streptococci and the growth of other organisms is inhibited, the following method is adopted with any cow which is exhibiting signs of clinical mastitis at the time of sampling. The sample is taken in the normal manner and the lid replaced *before* discarding the excess milk. The jar is inverted so that the absorbent pad is soaked with milk and any clots, the lid is removed and the excess milk poured off. The jar is specially marked to indicate that it comes from a cow with clinical mastitis. On receipt at the laboratory, a strong loop or spade is scraped along the surface of the absorbent pad and the material obtained is inoculated on to a blood-plate. By this means it is possible to recover those organisms which are inhibited by the selective medium. It is important to recognise that since the causal organism may be inhibited by the selective medium, there is a danger that a sample from a clinical case of mastitis may be reported as negative. In a comparative trial in which duplicate samples from clinical cases were taken into MacCartney bottles and on to the sample-jars there was agreement in 48 out of 50 cases. A noteworthy feature was the absence of contamination in the blood-plates which were inoculated from the absorbent pad.

SUMMARY

A method is described for the examination of milk samples for streptococci by direct sampling on to the surface of a selective medium. Screw capped jars with an absorbent pad are used, and in clinical cases material is also taken on the pad. The results obtained by this method have been compared with those secured by plating 0·1 ml. of milk on to blood agar and by streaking Edwards medium with gravity cream after preliminary incubation of the milk. For the final identification of *Str. agalactiae* a modification of the method first described by Christie and his co-workers has proved useful.

The introduction of this method has resulted in the number of samples examined at the laboratory being quadrupled while the cost of examining individual samples has been reduced to one quarter of the previous figure.

REFERENCES

Christie, R., Atkins, N. E., and Munch-Petersen, E. (1944). *Aus. J. exp. Biol.*, **22**, 197.

Maxted, W. R. (1948). *Lancet*, Aug. 14th, 255.

Munch-Petersen, E., Christie, R., and Simmons, E. T. (1945). *Aus. J. exp. Biol.*, **23**, 198.

Neave, F. K., Taylor, M. E., and Lawes, L. F. (1949). *Proc. Soc. appl. Bact.*, **1**, 34.

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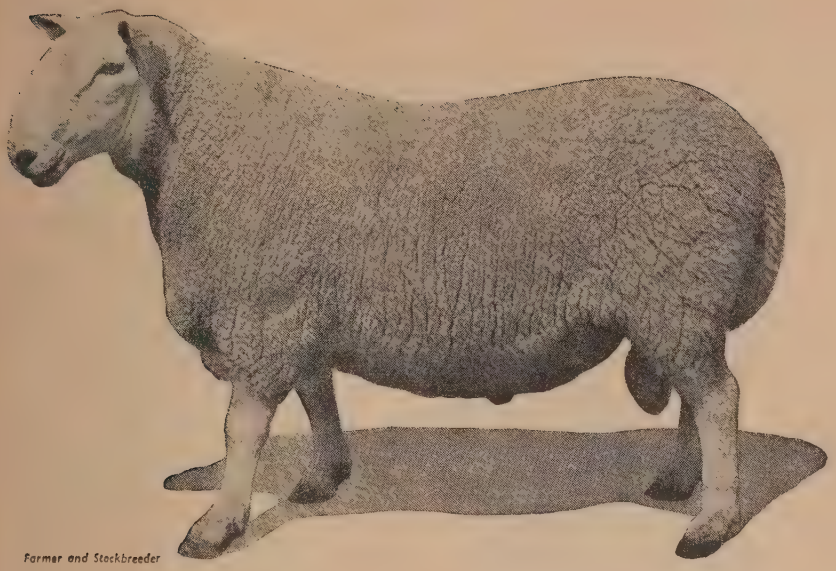

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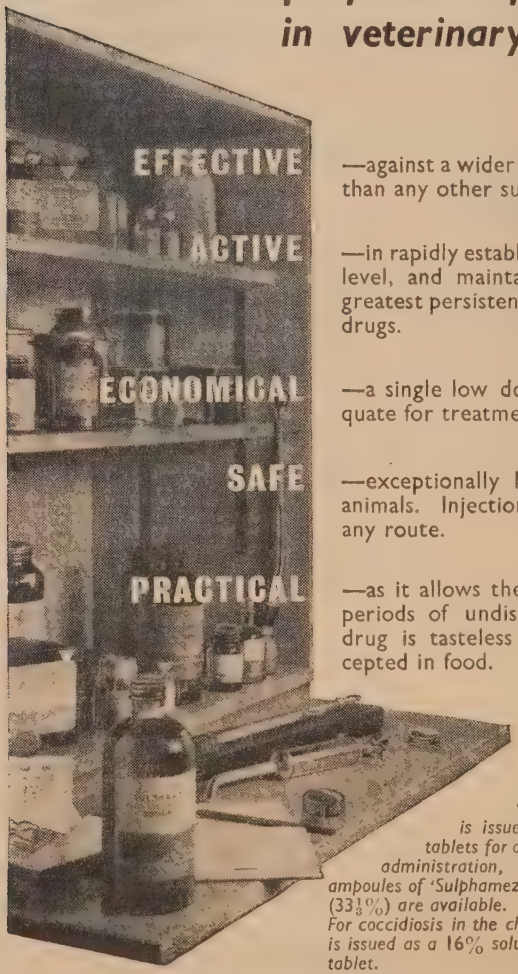

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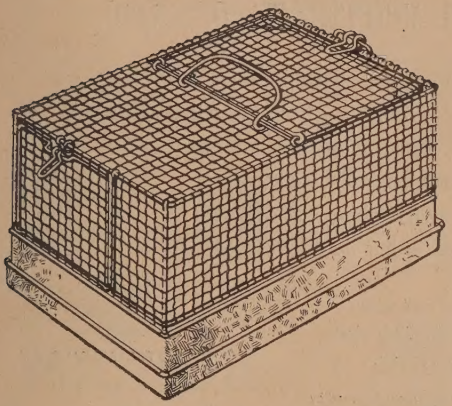

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The Block File stores paraffin blocks with utmost economy of space
The Slide Dryer dries slides in seven minutes
The Laboratory Slide Trays and Cabinet
The Slide-files for all types of slides and transparencies
The standardized reagents for tissue processing and staining
The electric Paraffin Knife for cutting, blocking and mounting tissues
The Mobile Refrigerator for gathering and storing tissues

an integrated system for every phase of histologic tissue preparation and reference

Autotechnicon

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automatic tissue processing by any histologic technique

The Autotechnicon System, as employed in a typical hospital working day, is interestingly discussed in an informative brochure. A copy will be sent on request to . . .

In thousands of hospitals, the world over, the benefits of the Autotechnicon System of mechanized tissue-processing procedures are being demonstrated daily. Production is improved, time saved, efficiency heightened, labor conserved. The Autotechnicon System is unique . . . the outgrowth of decades of intimate study of, and experience with, the technical problems of the pathologic laboratory.

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